TREATMENT OF INDUSTRIAL EFFLUENTS USING MIXED CULTURE OF MICROORGANISMS IN A BIOREACTOR

A thesis submitted in partial fulfilment of the requirements for the degree

of

Master of Technology

In

Biotechnology and Medical Engineering

By

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NATIONAL INSTITUTE OF TECHNOLOGY, ROURKELA
2010

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CERTIFICATE

This is to certify that the work in thesis entitled "**Treatment of industrial effluents using mixed culture of microorganisms in a bioreactor**" submitted by **Mr. Debadatta Das**, in partial fulfilment of the requirements for the award of Master of Technology in Biotechnology and Medical Engineering, with specialization in '**Environmental Biotechnology'** at National Institute of Technology, Rourkela (Deemed University) is an authentic work carried out by him under my supervision and guidance.

To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University/Institute for the award of any Degree or Diploma.

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ACKNOWLEDGEMENTS

ish to express my sincere gratitude to my Supervisor, Dr. (Mrs.) S. Mishra, for her guidance, encouragement, patience, and cooperation throughout the course of this research. I have been very fortunate to be under her guidance.

I am highly indebted to the authorities of NIT, Rourkela for providing me various infrastructures like library, computational facility and Internet access, which have enabled me to complete my research work successfully.

My sincere gratitude and appreciation goes to my dear friends, for being there whenever I needed them. Thank you very much Rajdeep, Shuchismita, Madhusmita, Vamsi Krishna, Akalabya and Satya for continuous encouragement.

Finally, I am forever indebted to my parents and my sister for their understanding and encouragement and support throughout every endeavour of mine. I have dedicated my work exclusively to them.

Debadatta Das

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ABSTRACT

Textile mill present in Rajahmundry, AP, India is one of the industries which is polluting the nearby water bodies and lands as well as the whole environment. Phenol and Chromium are the two major pollutants present in the effluent besides many organic, inorganic and heavy metal contaminants. Because of the hazardous nature, toxicity and exposure, Cr(VI) and Phenol have been designated as priority pollutants. There are many physical and chemical treatment methods available for the removal of both phenol and chromium but all these methods have problems associated such as secondary effluent, hazardous and harmful end products, high energy consuming, non economic etc. These problems can be overcomed by the use of biological treatment methods which are simple as well as eco-friendly and economic processes where complete removal of the pollutants is possible.

In this study simultaneous removal of phenol and Cr(VI) was achieved with the help of two indigenous microorganisms *Pseudomonas putida* (MTCC 1194) and *Escherichia coli* (NCIM 5051). The *Pseudomonas putida* (MTCC 1194) was capable of degrading phenol and the metabolites, energy and electrons produced during the phenol degradation is utilized by *Escherichia coli* (NCIM 5051) for the reduction of Cr(VI). The optimum condition was obtained for both the microorganisms as pH 7, temperature 30^0C, 12 hours inoculum age using 5% inoculums, shaking at 130 rpm with peptone and dextrose as nitrogen and carbon source. At optimum process condition individual *P. putida* could degrade maximum of 1000 ppm phenol and *E. coli* could degrade 40 ppm of Cr(VI) in shake flask culture. Simultaneous removal of phenol and Cr(VI) was examined with various combination of phenol and Cr(VI) concentrations when phenol was used as a carbon source. Simultaneous phenol and Cr(VI) degradation was studied at constant Cr(VI) concentration (5 mg/L) and varying the phenol concentration (50-1000) mg/L and vice versa. Simultaneous degradation of phenol and Cr(VI) was studied both using bioreactor and shake flask. Maximum degradation within less time was achieved using a bioreactor. The heavy metals resistance of the microorganisms shows the result in the sequence of Zn > Cu > Ni. The textile mill effluent was treated in a bioreactor at optimum conditions such as pH 7, temperature 30^0C, 12 hours inoculums age, 5% inoculums, agitation at 200 rpm and DO 100 mg/l. The effluent analyzed after treatment indicates that along with the major pollutant such as phenol and Cr(VI) other heavy metals also attained their permissible limits. The cytological and morphological changes that

occurred in the cells of the microorganisms both before and after treatment were observed. The mixed culture of microorganisms were capable of removing both the contaminants in one set of experiment that made the approach cost effective, time shaving. The simultaneous utilization of toxic intermediates was possible through the method. The studied indigenous organisms can be very well applied for the treatment of effluents containing the multiple contaminants such as phenol and chromium.

(Keywords: industrial effluent, chromium, phenol, *Pseudomonas putida*, *Escherichia coli*)

1. INTRODUCTION

Waste water released from various industries is the major concerns for environmentalists now days. Industrial effluents contain various toxic metals, harmful gases, and several organic and inorganic compounds. Both the quality and quantity of effluent result in various impacts on the availability of good quality water as well as on marine environment. Due to the discharge of these toxic effluents, there has been a major loss in the ecological, social and economic perspective. The long-term consequences of exposure also cause fatal diseases like cancer, delayed nervous responses, mutagenic changes, neurological disorders etc [Wahaab, (2000) and Balaji et al., (2005)].

Chromium metal is an example of a heavy metal found in the effluents of industries, such as in metal finishing, petroleum refining, iron and steel industries, textile manufacturing, electroplating, leather tanning metal plating etc [Ramakrishna and Philip, (2005); Horton et al., (2006); Das and Mishra, (2010); Poornima et al., (2010)]. Effluents of these industries contain large quantities of chromium-laden in their wastewater. Hexavalent chromium (Cr(VI)) compounds are considered to be highly toxic, carcinogenic, and mutagenic to living organisms [Poopal and Laxman, (2009) and Ramakrishna and Philip, (2005)]. But trivalent chromium (Cr(III)) is generally toxic only to plants at very high concentrations and is less toxic or non-toxic to human and animals [Shen and Wang, (1994), Megharaj et al., (2003)]. Because of its hazardous nature, toxicity and exposure, Cr(VI) has been designated as a priority pollutant in many countries [Wang and Xiao et al., (1995); Shakoori et al., (2000); Megharaj et al., (2003)]. Due to the high toxicity of chromium the central pollution control board has fixed the minimum permissible limits of Cr(VI) and total chromium at 0.1 mg/l and 2 mg/l respectively.

Phenol is another major pollutant included in the list of EPA (1979). Aqueous phenolic effluents are relatively common industrial wastes, being produced in several industries and operations such as petroleum refineries, gas and coke oven industries, phenolic resins, explosive manufacture, plastic and varnish industries, textiles units using organic dyes, and smelting and related metallurgical operations [Mahadevaswamy et al., (1997); Bandyopadhyay et al., (1998); Jayachandran and Kunhi, (2008); Marrot et al., (2006); Paula and Young, (1998)]. Acute exposure of phenol causes central nervous system disorders. Acute exposure of phenol can result in myocardial depression. Phenol causes a burning effect

on skin. It can also cause hepatic damage [Kumar et al., (2005); Bandyopadhyay et al., (1998); Annadurai et al., (2007); Arutchelvan et al., (2006)]. In these perspectives central pollution control board set the minimum permissible level for phenol in environment as 0.05 to 0.1mg/l.

Chromium and aromatic compounds like phenol are discharged together in industrial processes such as wood preserving, metal finishing, petroleum refining, leather tanning and finishing, paint and ink formulation, pulp and paper industry, Textile Industry Pharmaceutical industry and manufacturing of automobile parts [Song et al., (2009); Tziotzios et al., (2008); Wang and Chirwa, (1998); Yun-guo et al., (2008); Nkhalambayausi-Chirwa and Wang, (2001)]. Traditionally, high concentrations of Cr(VI) and phenol in industrial wastewater require the use of expensive chemical and physical processes for their removal like ozonization, adsorption, ion exchange, membrane filtration, chemical oxidation etc [Aksu and Gonen, (2006; Tziotzios et al., (2008); Wang and Chirwa, (1998); Yun-guo et al., (2008)]. But these process are high energy consuming, non economic and release effluents and waste waters which again pollute the environment. To overcome the drawbacks of these physical and chemical methods recently biotechnological processes have been reported as an alternative to these complex and expensive treatment methods. Biological methods are simple as well as eco-friendly processes and have the potential to completely reduce and degrade the pollutants under aerobic or anaerobic conditions at relatively low capital and operating cost.

Very few researchers have initiated research work on degradation of phenol and chromium in mixed culture of different phenol reducing and chromium degrading microorganisms. There have been very few reports on degradation using indigenous microorganisms such as *P. putida* MTCC (1194) and *E. coli* NCIM (5051) for the simultaneous removal of phenol and chromium from textile industry effluent and also bringing the other heavy metals present in the effluent to below their permissible limits. There is lack of a complete investigation on maximum degradation level of both the pollutants when occurring simultaneously in presence of mixed culture of organisms in shake flask and bioreactor. Moreover there are only a few reports on the morphological and cytological changes that take place in the cell wall during the degradation process. Hence degradation studies using indigenous microbes have been sorted to treat Cr(VI) and phenol in the textile industry effluent.

2. LITERATURE REVIEW

Pollution of water by industrial effluents of process industries is a serious problem in most countries. Industrial waste consists of both organic and inorganic substances. Organic wastes include pesticide residues, various hydrocarbons, solvents, cleaning fluids, dissolved residue from fruit and vegetables, and lignin from pulp and paper etc. Effluents can also contain inorganic wastes such as brine salts and metals. The increased industrial activities have reduced the availability of good quality water by producing a large amount of effluents to the rivers. Industrial effluents often contain various toxic metals, harmful gases, and several organic and inorganic compounds [Balaji et al., (2005)]. Due to discharge of toxic effluents long-term consequence of exposure can cause cancer, delayed nervous damage, malformation in urban children, mutagenic changes, neurological disorders etc [Govindarajalu, (2003)]. Phenol and chromium are the major contaminant present in the effluent discharged from the various industrial processes such as wood preserving, metal finishing, petroleum refining, leather tanning and finishing, paint and ink formulation, pulp and paper industry, Textile Industry Pharmaceutical industry and manufacturing of automobile parts industries [Song et al., (2009); Tziotzios et al., (2008); Wang and Chirwa, (1998); Yun-guo et al., (2008); Nkhalambayausi-Chirwa and Wang, (2001)].

2.1. Phenol

Phenol is a White Crystalline Solid. It contains a six-membered aromatic ring, bonded directly to a hydroxyl group (OH) having chemical formula C_6H_5OH [Prpich and Daugulis, (2005)]. Phenol is a hygroscopic [Collins and Daugulis, (1997)], slightly acidic by nature. It has a distinctive odour. Its molecular weight is 94.11, density is 1.072 and the boiling point is 181.9°C. Its other names are Carbolic acid, Benzenol, PhenylicAcid, Hydroxybenzene, Phenic acid [Annadurai et al., (2000)].

2.1.1. Uses of phenol

- Medical Use: Phenol has antiseptic properties and is used for aseptic (germ-free) techniques in surgery and sanitation purposes.
- Industrial Use: Aqueous phenolic effluents are relatively common industrial wastes, being produced in several industries and operations such as herbicides, and synthetic

resins industries petroleum refineries, gas and coke oven industries, phenolic resins, explosive manufacture, plastic and varnish industries, textiles units using organic dyes, and smelting and related metallurgical operations etc [Mahadevaswamy et al., (1997); Bandyopadhyay et al., (1998); Marrot et al., (2006); Bodalo et al., (2008); Jayachandran and Kunhi, (2008)].

- Laboratory Use: Phenol is used along with chloroform (a commonly-used mixture in molecular biology for DNA & RNA purification from proteins) and also used for cell disruption and lysis purpose.
- Beauty Products: Phenol is also used in the preparation of cosmetics including sunscreens, hair dyes, and skin lightening products.

2.1.2. Toxicity of phenol

Phenol is a major pollutant included in the list of EPA (1979) as reported by Agarry et al., (2008). Acute exposure of phenol causes disorders of central nervous system. Hypothermia, myocardial depression, burning effect on skin, irritation of the eyes, also it causes gastrointestinal disturbance are same of the effects reported by the researchers [Tziotzios et al., (2005) and Chakraborty et al., (2010)]. As per the rules of central pollution control board the minimum permissible level for phenol in environment is 0.1mg/l [Kumaran and Paruchuri, (1996); Nuhoglu and Yalcin, (2005) and Saravanan et al., (2008)].

2.2. Chromium

Chromium is a chemical element discovered in 1797 by Louis Nicolas Vauquelin which has the symbol Cr. Its atomic number is 24. It is a hard metal of steely gray colour and also it has a high melting point of 1907°C. It is odourless and tasteless metal. Many of its compounds are intensely coloured. Chromium is important metal due to its high corrosion resistance and hardness. The trivalent chromium (Cr(III)) is required in trace amounts for sugar and lipid metabolism in humans and its deficiency causes disease. Hexavalent chromium (Cr(VI)) is a highly toxic metal pollutant that affects the environment and is abundantly available in the environment. Due to its toxicity and harmful effect on living system its cleanup is highly essential.

2.2.1. Uses of chromium

Chromium metal is found in the effluents of industries, such as in metal finishing, petroleum refining, iron and steel industries, textile manufacturing, electroplating, leather tanning metal plating etc [Shen and Wang, (1994); Horton et al., (2006); Das and Mishra, (2010)]. Effluents of these industries contain large quantities of chromium-laden in their wastewater [Poornima et al., (2010)]**.** Hexavalent chromium Cr(VI) compounds are considered to be highly toxic, carcinogenic, and mutagenic to living organisms [Ramakrishna and Philip, (2005) and Poopal and Laxman, (2009)]. Considering its potential for hazardous toxicity and exposure, Cr(VI) has been designated as a priority pollutant in many countries. [Bae et al., (2000); Cheunga and Gu, (2007); Srivastava et al., (2008)].

2.2.2. Toxicity of chromium

Considering its potential for hazardous toxicity and exposure, Cr(VI) has been designated as a priority pollutant in many countries. Chromium(VI) is toxic and harmful to human health, mainly for the people who are working in industries where Cr(VI) is widely used . Chromium (VI) causes various health problems to human beings as reported by [Dermou et al., (2005)]. It has been reported by various authors that hexavalent chromium causes lung cancer **,** ulcer, severe damage to the liver and kidneys, perforation of nasal septum, leukocytosis, Skin rashes in humans [Poornima et al., (2010); Pechova, (2007); Kaufman et al., (1970); Li et al., (1987)]. There is sufficient evidence for carcinogenicity of Cr(VI) in animals for the hexavalent chromium compounds like calcium chromate, chromium trioxide, lead chromate, strontium chromate and zinc chromate stated by Poopal and Laxman, (2009). IARC and ACGIH has also classified chromium metal and trivalent chromium compounds as not human carcinogen. According to central pollution control board the minimum permissible limits of Cr(VI) at 0.05-0.1 ppm and 2 ppm for total chromium is sited [Das and Mishra, (2010)].

2.3. Different Physical and chemical Methods for phenol and chromium degradation and their draw backs

Traditionally, by the use of various expensive chemical and physical processes high concentrations of both Cr(VI) and phenol are reduced from the industrial wastewater. Those methods include ozonization, adsorption, ion exchange, membrane filtration, chemical oxidation etc [Arutchelvan et al., (2006); Aksu and Gonen, (2006); Meghraj et al., (2003); Das and Mishra, (2010)]. But these process are high energy consuming, non economic and release effluents and waste waters which requires further treatment and thus are alarming for the environment. Also complete removal of the pollutants cannot be possible by the use of physical and chemical processes [Camargo et al., (2003); Shen and Wang, (1994); Wang and Chirwa, (1998)].

2.3.1. Ozonization

Chemical oxidation with ozone can be used to treat organic pollutants or act as disinfectant agents. Ozone is a powerful oxidant that can oxidize a great number of organic and inorganic materials. Ozone based technologies research is also being focused on the catalytic ozonation where the presence of catalyst significantly improved the oxidation rate of organic compounds compared to non catalytic ozonization. The ozonization processes are possibly one of the most effective methods for treatment of wastewater containing organic products such as effluents from chemical and agrochemical industries, textile industry, paints, etc. [Guendy, (2007)]. However, the characteristics of the wastewater such as pH, temperature and concentration of organic pollutant play an important role in organic degradation [Yogeswary et al., (2005)]. Similarly [Wang et al., (2008)] have studied the treatment of industrial effluent by treatment with ozone initial pH, ozone dosages and dosages of catalyst on effluent treatment. Then, organic matter remained was removed by BAF(Biological aerated filter), and the effect of hydraulic retention time (HRT) of BAF was also considered. Their result showed that under optimum pH and dose of H_2O_2 was about 30% COD could be removed by ozonization alone. The use of high oxidation potential like ozonization has recently received much attention in wastewater treatment studies. Ozonizer in which the oxygen molecules in the gas were dissociated to form ozone was used in many cases [Matheswaran et al., (2007)]. The disadvantages associated with the process are high

operating cost. The cost of the equipment is very high and also it requires high voltage and electricity for its operation [Gharbani et al., (2010)].

2.3.2. Ion exchange

Ion exchange means the removal of an ion from an aqueous solution by replacing another ionic species. There are natural and synthetic materials available which are specially designed to enable ion exchange operations at high levels. So ion exchangers are used to perform this ion exchange for removal of organic and inorganic pollutants along with other heavy metals for purification and decontamination of industrial effluent. Synthetic and industrially produced ion exchange resins are mainly made up of polystyrene and polyacrylate are in the form of small and porous beads. Also there are some naturally occurring minerals which have ion exchange properties. The most common one is aluminium silicate minerals which are also called zeolites [Raghu and Basha, (2007)]. There are different zeolites available made up of various ionic materials which have affinity towards some particular metals. The main features of the ionic resins include material properties such as like adsorption capacity, porosity, density etc [Zorpas et al., (2010)]. The main disadvantages associated with ion exchange method are the high cost of the ion exchange resins and each resin must be selectively removes one type of contaminant only. Further, complete removal of the contaminant is not possible. Besides, it can be used for limited cycles only as by passing concentrated metal solution the matrix gets easily owned out by organics and other solids in the wastewater after several use. Moreover ion exchange is also highly sensitive to pH of the solution [Liotta et al., (2009) and Sapari et al., (1996)].

2.3.3. Adsorption

Adsorption is a widely used method for the treatment of industrial wastewater containing colour, heavy metals and other inorganic and organic impurities stated by Al-Rekabi et al., (2007) and Patel and Vashi., (2010). Adsorbent materials are basically derived from low-cost agricultural wastes, activated carbon prepared from various raw materials such as sawdust, nut shells, coconut shells etc Zawani et al., (2009). These adsorbents are basically used for the effective removal and recovery of organic and metal pollutants from wastewater streams [Basso et al., (2002) and Park et al., (2006)]. It is a complex process affected by several factors. Mechanisms involved in the adsorption process mainly focus on the selection of the

adsorbent material like their particle size surface area and porosity etc [Gardea-Torresdey et al., (2004)]. This method suffers from low adsorption capacity and in some cases complete removal is not possible and high cost of the adsorbent. After use the disposal of adsorbants creates problems.

2.3.4. Membrane filtration

Membrane filtration technique has received a significant attention for the wastewater treatment. It considers the application of hydraulic pressure to bring about the desired separation through the semi permeable membrane [Chen et al in 2004]. Membranes are of different pore size and it is necessary to select membranes of appropriate pore size for specific purpose so that effluent and wastewater could be purified and permeate could be recycled a number of times. Mainly three types of membrane filtration is there. They are Ultra-filtration, Nanofiltration and Reverse osmosis reported by Chauhan and Rekha, (2004) and Al-Rekabi et al., (2007). Various other types of membranes such as inorganic, polymeric, and liquid membranes can be used in this process. The main problem associated with this process is incomplete removal of contaminants, high energy requirement, high cost of the membrane and longevity of the membrane. After long term use the membrane get clogged with the contaminants present in the waste water and is damaged due to extra pressure on the membrane.

2.3.5. Chemical oxidation

In this process the waste materials from the industrial waste water are removed by the help of chemical oxidation by the use of various chemicals mainly hydrogen peroxide is widely used for this purpose as reported [Dias-Machado et al., (2006) and Ksibi, (2006)]. There are many disadvantages associated with this process like the high cost of the chemicals, emission of various harmful by products, it creates hazardous constituent like secondary effluent problem along with the production of harmful gases.

2.4. Biodegradation

Biodegradation is the process of decaying or reduction of different organic materials and toxic metals to their non toxic form with the help of microorganisms. In this process complete

mineralization of the starting compound to simpler ones like CO_2, H_2O, NO_3 and other inorganic compounds takes place [Atlas and Bartha, (1998)].In the mixed culture of microorganisms phenol degrading organisms utilizes phenol as sole source of carbon and produce energy, metabolites, electron donor which is used by the chromium degrading organisms to reduce chromium [Yun-guo et al., (2008); Tziotzio et al., (2008) and Song et al., (2009)]. Biodegradation is a microbial process in which nutrients and physical conditions plays important role. Temperature and pH are the important physical variables and carbon, nitrogen, oxygen, phosphorus, sulfur, calcium, magnesium, and several metals are the micronutrients that also shows a significant impact on degradation behavior is reported [Khazi et al., (2007)].

2.4.1. Advantages of biodegradation

There are various advantages associated with biodegradation such as
The process is a simple process. It is an eco-friendly and cost effective process that requires low capital and operating cost. Being environmentally friendly process it produces no harmful end products

2.4.2. Microorganisms involved in biodegradation of phenol and chromium

Degradation of phenol occurs as a result of the activity of a large number of microorganisms including bacteria and fungi. Bacterial species include *Bacillus sp, Pseudomonas sp, Acinetobacter sp, Achromobacter sp etc. Fusarium sp, Phanerocheate chrysosporium, Corious versicolor, Ralstonia sp, Streptomyces sp* etc are also proved to be efficient fungal groups that evidenced phenol biodegradation [Chitra et al., (1995);Nair et al., (2008); Basha et al., (2010); Kumar et al., (2005); Mordocco et al., (1999); Chung et al., (2003); Santos et al., (2006); Chen et al., (2003)].
Similarly chromium degradation can be possible with the help of variety of bacteria and fungal groups like *Arthrobacter* sp. and *Bacillus* sp., Megharaj et al., (2003), E. coli ATCC 33456 [Bae et al 2000 and Shen et al 1994], *Pseudomonas aeruginosa* [Aguilera et al., (2004)], *Brevebacterium casei* [Das and Mishra, (2010)], *Acinetobacter sp* [Srivastava et al., (2007)] some unidentified species like *Pseudomonas* fluorescens, *Pseudomonas* synxantha [Gopalan and Veeramani, (1994), McLean et al., (2000)] *Alcaligenes eutrophus* Vaneechoutte et al., (2004) A number of bacterias Bacillus spp., Shewanella alga BrY-MT

and a few unidentified strains have also been shown to reduce Cr 6^{+} [Shen and Wang, (1994), Wang and Xiao, (1995); Shakoori et al., (2000); Guha et al., (2001); Camargo et al., (2003)].

2.4.3. Mechanism of biodegradation

- Most of the efficient phenol degrading microorganisms are capable of using phenol as the sole source of carbon and energy for their cell growth and metabolism.
- Microorganisms capable of degrading Phenol through the action of variety of enzymes.
- Phenol is first converted to catechol by the help of hydroxylase enzyme, the catechol is degraded via ortho or meta cleavage of central metabolism.
- By which the enzyme present in the microorganisms i.e catechol 2, 3 dioxygenase that cut the ring at the meta position.
- Another enzyme is also present in the microbes i.e catechol 1, 2 dioxygenase cut the benzene ring at the ortho position benzene ring.
- The benzene ring is opened through meta oxidation. The catechol is converted to other intermediates trough ortho and meta cleavage. These intermediates are finally consumed by the microbes with the help of various enzymes through the TCA cycle resulting CO_2, metabolites and energy. [Nair et al., (2008), Aggary et al., (2008), Basha et al., (2010)].
- *E. coli* utilizes the metabolites formed from phenol degradation as electron donors and energy for the reduction of Cr(VI). By this biological mechanism *E. coli* can directly transform the highly toxic Cr(VI) is to less toxic Cr(III) with the help of various membrane associated, NADH dependent soluble chromate reductase enzymes [Bae et al., (2000), Cheunga et al., (2007), Wang and Shen, (1993), Shen and Wang, (1994)].

$$\text{Phenol + Oxygen} \xrightarrow[\text{Oxidation}]{\textit{P. putida}} \text{Energies + metabolites + Electrons}$$

$$Cr^{6+} + \text{Electrons} \xrightarrow[\text{Reduction}]{\textit{E. coli}} Cr^{3+}$$

Different process variables like pH, temperature, inoculums volume, media composition etc and their influence on phenol degradation was reported by different authors. Maximum concentration of phenol degradation by different authors using different microorganism was also studied.

Media composition along with selection of best carbon and nitrogen source is a very important parameter to be studied various researchers. They have studied the degradation efficiency under different carbon and nitrogen sources. Ramakrishna and Philip, (2005) and Das and Mishra, (2010) suggest the effect of different electron donors on Cr(VI) reduction using *Ganoderm lucidum* and *Brevibacterium casei* respectively. Among several electron donors and nitrogen sources screened they concluded dextrose was the optimum electron donor and peptone showed maximum Cr(VI) reduction. Similarly maximum degradation of Cr(VI) evidenced in the presence of dextrose by Bae et al., (2000) by using *E. Coli* ATCC 33456. Also Kotresha and Vidyasagar, (2008) achieved 100% phenol degradation using *P. aeruginosa* MTCC 4996 with peptone and dextrose as nitrogen and carbon source respectively. But Suhaila et al., (2010) achieved maximum phenol degradation by using *Rhodococcus* UKM-P in the presence of ammonium sulphate. From the media optimization study it is concluded that different carbon and nitrogen sources are required for the degradation of various pollutants by different microorganisms.

Phenol degradation under different pH range was studied by various authors. Khazi et al., (2007) have studied the phenol degradation at different initial range of pH values like 4, 5, 6, 7 and 8. From the result it was found that the initial pH greatly influences the growth and degradation rate. Among the various pH values, the pH 7 was found to produce more amounts of cell mass and evidenced better degradation of phenol. Similarly Veenagayathri and Vasudevan, (2010) have examined the effect of pH on 100 mg/L phenol degradation by the bacterial consortium under different pH like (5.5, 6, 6.5, 7, 7.5, 8 and 8.5). The maximum phenol degradation efficiency of 99 % was achieved at pH 7. Maximum adsorption and degradation capacity of *Pseudomonas pictorium* (ATCC 23328) was investigated by Annadurai et al., (2007) by immobilizing with chitin under various range of pH (7 to 9) and maximum result was obtained at pH 7.0. But maximum degradation of phenol at optimum process condition of pH at 7.5 was reported by Suhaila et al in 2010 using *Rhodococcus* UKM-P, in shake flask culture. Similarly Arutchelvan et al., (2006) have reported maximum phenol degradation using *Bacillus brevis* at pH 8.0. Bae et al., (2000) have evaluated the

optimum Cr(VI) reduction by *E. coli* ATCC 33456 within a pH range of (3-8) and the optimum pH reported was 7. Similarly Cr(VI) reduction in E. coli ATCC 33456 was evaluated within a pH range of (3–8) by Shen and Wang, (1994) and the result showed maximum initial specific rate of Cr(VI) reduction at pH 7. Also they have reported that effect of pH on Cr(VI) reduction were similar for both aerobic and anaerobic cultures. Das and Mishra, (2010) used *Brevibacterium casei* for degradation of chromium.They observed the reduction of Cr(VI) using wide range of pH ranging from pH 4 to pH 9 and the maximum degradation was reported at pH 7. The above studies conformed that pH has a significant influence on phenol as well as chromium degradation.

Similarly temperature also has a great influence on the degradation of phenol and chromium. Many researchers have tried to investigate the effect of temperature on the rate of degradation. Hank et al., (2010) examined the effect of temperature on the degradation of phenol by *Pseudomonas aeruginosa* at 30°C and 40°C and concluded optimum temperature at 30°C. Similarly Khazi et al., (2007) also reported the complete degradation of phenol at 30^0C. Bandyopadhyay et al., (1998) studied the kinetics of phenol degradation using *P. putida* MTCC 1194 in the presence of different temperature variables. The optimum temperature required for maximum phenol biodegradation was found at 30°C. But Zeng et al., (2010) used two newly isolated high phenol-degrading strains of bacteria *Micrococcus* sp. and *Alcaligenes faecalis* JH 1013, and the optimal conditions for phenol degradation were reported at temperature 32^0C. Similarly Bajaj et al., (2009) reported maximum phenol degradation at 25^0C by mixed bacterial consortium. Arutchelvan et al., in (2006) have reported that using *Bacillus brevis* maximum phenol degradation at 34^0C was achieved. Bae et al., (2000) studied Cr(VI) reduction using *E. coli* ATCC 33456 in the temperature range of 40^0C to 60^0C and found optimum value at 37°C. Temperature range of 10^0C to 45^0C was studied by Shen and Wang, (1994) and the temperature of 36^0C was found to be optimum. Thus results suggested that temperature has a great influence on both phenol and chromium degradation. The temperature is a very important factor effecting the degradation as it is a enzymatic process where the enzymes present in the microorganisms show maximum functionality at a certain temperature.

Phenol and chromium degradation were also examined at different age and volume of inoculums by various researchers. Khazi et al., (2007) reported that the most suitable inoculums age for highest phenol degradation by *P. putida* MTCC1194 was 12 hours at 5% of inoculum volume. Bae et al., (2000) have also demonstrated that 12 hours old and 5%

inoculums volume were optimum for Cr(VI) degradation in *E. coli* ATCC 33456. The various amounts of inocula like 0.5%, 1%, 2%, 3%, and 5% (v/v) were used in experiments on Cr(VI) reduction and phenol degradation by Song et al., (2009) and optimum degradation was reported at an inoculation volume of 5%. This shows the inoculums age and volume are the important factors affecting phenol degradation and chromium reduction. It is established that microorganisms subjected to degradation should be in its active phase. If it is in the death phase it will show a longer lag phase resulting increase in time taken for degradation and also the degradation efficiency will be greatly reduced.

2.5. Degradation of Phenol and Chromium(VI) using individual species

Various researchers have studied the individual and simultaneous phenol and chromium reduction at their optimum process parameters. Khazi et al., (2007) reported 750 ppm phenol degradation in 60 hours using *P. putida* MTCC1194 under optimum conditions like temperature 30^0C, pH 7, inoculum volume 5%, 12 hour grown culture. Similarly Bandyopadhyay et al., (2000) reported 1000 ppm of phenol degradation using *P. putida* MTCC1194 under optimum conditions like temperature 30^0C, pH 7, inoculum volume 7%, 12 hour grown culture. Arutchelvan et al., (2006) reported maximum 1750 ppm of phenol in 144 hours at optimum process condition of pH 8.0, temperature 34 ± 0.1^0C and 5% of inoculums volume. Kotresha and Vidyasagar, (2008) reported degradation of maximum 1300 mg/l phenol degradation in 156 hours by *Pseudomonas putida* at optimum temperature of 37^0C and pH 7. Similarly Annadurai et al., (2002), reported that the optimum growth conditions for complete degradation of phenol using *P. putida* (ATCC31800) was reported at pH 7.0, temperature 30^0C in presence of glucose and yeast extract as carbon and nitrogen source respectively. Kumar et al., (2005) also reported the maximum degradation of 1000 ppm phenol in 162 hours using *P. putida* MTCC 1194 under the optimum process variables of pH 7.1 and temperature at 30^0C. Bae et al., (2000) obtained maximum 100% to 84% Cr(VI) degradation for the range of (10 to 40) ppm concentration in the influent using *E. coli* ATCC 33456. Similarly Shen and Wang, (1994) also observed the maximum of 30 mg/l Cr(VI) degradation in 45 hours at optimum condition of pH 7, temperature 36^0C, and glucose as a electron donor. Under optimum process conditions of pH 7, temperature 30^0C, inoculums volume 1.5%, dextrose and peptone as carbon and nitrogen source respectively reported maximum degradation of 50 ppm Cr(VI) in 12 hours by using *Brevibacterium casei* [Das and Mishra, (2009)].

2.6. Simultaneous phenol and Cr(VI) degradation using mixed microorganisms

There have been a number of literatures available for individual degradation of phenol and chromium, however very few researchers have initiated research work for simultaneous phenol and chromium degradation using mixed culture of different phenol degrading and chromium reducing microorganisms. This is a cost effective, eco-friendly and time shaving approach towards the complete removal of more than one pollutants present in the waste water. Cost effective in the sense one time media utilization gives rise to degradation of both the pollutants at a time. It is an eco-friendly approach like the use of single microorganism can produce toxic intermediates by the degradation of the pollutants. By the use of mixed culture of microorganisms this problem can also be overcome like the metabolites produced from the degradation of one pollutant by a particular organism can be used by the another microorganism as energy sources for the removal of another pollutant. It is time shaving method because in the same time both the pollutants could be removed from the waste water. In the simultaneous phenol degradation and chromium reduction study the phenol degrading organism degrades the phenol while producing electron donors, matabolites and energy which was used by the chromium reducing organisms who accepts the electrons being produced by the phenol degradation and use them to reduce the hexavalent chromium to less toxic trivalent chromium. A few researchers have investigated the simultaneous phenol and chromium reduction under optimum process variables. Yun-guo et al., (2008) studied the simultaneous removal of Cr(VI) and phenol in a coculture containing chromium reducer, *Bacillus sp*. and phenol degrader, *Pseudomonas putida Migula* CCTCC AB92019. In the above study phenol was used as the sole carbon source. The *Bacillus sp*. utilized metabolites formed from phenol degradation as electron donors and energy source for reduction of Cr(VI). Optimum Cr(VI) at an initial concentration of 15 mg/L was observed when the phenol concentration was 150 mg/L in 60 hours under optimum process parameter of pH 7 and temperature of 30^0C. Their study revealed that the toxicity of Cr(VI) had a inhibiting effects on phenol degradation, whereas phenol enhanced Cr(VI) reduction under the optimum level. Similar study carried out by Wang and Chirwa et al, (1998) studied the simultaneous removal of the two pollutants by using a phenol degrading organism *Pseudomonas putida* DMP-1 and a Cr(VI) reducing strain *Escherichia coli* ATCC 33456, with phenol as the sole added carbon and energy source. From the study it was assumed that metabolites formed from phenol degradation were used by *E. coli* for Cr(VI) reduction. Nkhalambayausi-Chirwa and Wang, (2001) also examined the similar work and observed the optimum Cr(VI)

reduction was at a phenol concentration of 200mg L^{-1} and an initial Cr(VI) concentration of 2mg L^{-1}, while complete phenol degradation was observed in cultures only under low initial Cr(VI) concentrations (≤10mgL^{-1}). The result indicated complete Cr(VI) reduction and phenol degradation was observed at loadings of (5–21) mg Cr(VI) d^{-1} L^{-1} and (840–3350) mg phenol $d^{-1}L^{-1}$. The experimental results also indicated that metabolites formed from phenol degradation were utilized by *E. coli* as electron donors for reduction of Cr(VI). Similar finding were also demonstrated by Tziotzios et al., (2008), where the maximum of 3.574 g L^{-1} d^{-1} of phenol degradation and 0.062 g L^{-1} d^{-1} of chromium reduction was attained under the optimum condition of temperature of 26 ± 1^0C and pH range 7.3 to 7.5. Song et al., (2009) investigated the simultaneous Cr(VI) reduction and phenol degradation using the pure culture of *Pseudomonas aeruginosa* CCTCC AB91095 where Phenol was used as carbon source. *P. aeruginosa* utilized metabolites formed during phenol degradation as energy source for Cr(VI) reduction. The inhibition effect for both Cr(VI) reduction and phenol degradation was observed when Cr(VI) concentration exceeded the optimum value (20 mg/L), and phenol concentration of 100 mg/L. Maximum degradation was observed in 72 hours while the optimum experimental conditions like 5% (v/v) inoculum after incubation for 12 hour, temperature 37^0C and pH 7 were maintained throughout the study.

2.7. Present study

There has been few reports on use of indigenous microorganisms such as phenol degrading *Psudomonas putida* (MTCC 1194) and chromium reducing *Escherichia coli* (NCIM 5051) for the simultaneous removal of phenol and chromium from industrial waste water. In the present study the main aim is to remove both phenol and chromium present in the textile industry effluent collected from Rajahmundry, Andhra Pradesh using mixed culture of microorganisms so as to bring the organic and inorganic contaminants along with other heavy metals present in the industrial effluent to its permissible limits. The main mechanism behind the study is to degrade the phenol with the help of the phenol degrading microorganism *P. putida* that produce the metabolites, energy and electrons donors during phenol degradation which is utilized by the chromium reducing microorganism *E. coli* to reduce the toxic hexavalent chromium to less toxic trivalent chromium.

2.8. Scope of the study

The present work primarily involves the study on biological degradation of phenol using bacterial strain of *Pseudomonas putida* (MTCC 1194) and chromium using *Escherichia coli* (NCIM 5051). The cultures of *P. putida* (MTCC 1194) and *E. coli* (NCIM 5051) were exposed to increasing concentrations of phenol and chromium respectively to acclimatize them to high concentrations of phenol and chromium respectively and to increase their potential of degradation. The different parameters in the degradation process like pH, Temperature, Carbon source, Nitrogen source and Inoculum volume were also optimized for individual organisms .These optimized conditions were used for degradation of phenol and chromium using individual and mixed culture of microorganisms in shake flask and bioreactor study. The metabolites formation was clearly observed by studying the cells under 100X microscope The optimized conditions obtained were also used to study the degradation of an industrial effluent and to bring down to its permissible limits. The elemental analysis of the untreated and treated effluent was also carried out by Energy Dispersive X-Ray Spectroscopy. The total chromium and other heavy metal content was analysed by using Atomic Absorption Spectroscopy. The structural changes of the microorganism before and after the degradation process were also studied with help of Scanning Electron Microscopy.

3. MATERIALS AND METHODS

3.1. Effluent collection and analysis

Industrial effluents were collected from a local textile industry situated in Rajahmundry, Andhra Pradesh, India. Then the sample was subjected for various analysis like BOD, COD, TOC, TDS, TSS. The physico-chemical parameters were estimated using standard methods. Characteristics and compositions of other heavy metals present in the textile industry effluent were studied.

3.2. Chemicals

Pure and analytical grade chemicals were used throughout the experiments including media preparation for growth. Peptone, yeast extract, beef extract and nutrient agar and nutrient broth were supplied by Merck chemicals, India. Chemicals used for analysis of Phenol and Chromium like Potassium dichromate and 1,5-Diphenylcarbazide, Phenol, 4-Amino anti pyrine, potassium ferricynide, Ammonia, Sulphuric acid, Acetone was procured from Merck, Chemical, India. The composition of the minimal salt media used for the study were Calcium chloride dihydrate, Di-Potassium hydrogen phosphate , Ferrus sulfate heptahydrate, Magnesium sulfate heptahydrate, Potassium dihydrogen phosphate, Sodium chloride, Ammonium sulphate.

3.3. Glassware and apparatus

All glass wares (Conical flasks, Measuring cylinders, Beakers, Petriplates and Test tubes, burette, pippetes etc.) are purchased from M/s SAN Medico Ltd (Rourkela, India) under the name Borosil. The instruments and apparatus used throughout the experiment are listed below in table 1.

Table 1: List of Instruments used during the whole experiment their make and function

Equipment	**Make and Model**	**Use**
Vertical Autoclave	Tomy	Sterilization
Analytical Balance	Sartorius	Weight Measurement
Laminar airflow	Zhichen (ZhJH-1109C)	Aseptic Environment
pH	EuTech Instruments	Measurement of pH
Temperature controlled incubator shaker	Reico	Incubation and shaking of cultures
Ultra Low Temperature freezer	New Brunswick (U410)	Preservation of cultures
Ultra pure water system	Milli Q	Preparation of solutions and stocks.
Spectrophotometer(UV/Vis)	Shimadzu UV-160A	Estimation studies
Centrifuge	REMI	Collection of pellet
Colony counter	Systronics	Cell count
Biofermenter	New Brunswick(BIO FLO 410)	Batch degradation study
TOC analyzer	Shimadzu	Estimation of Total organic carbon
SEM-EDX	JEOL JSM-6330F, Japan	Elemental analysis of sample
Low Temperature freezer	LG	Preservation of cultures
AAS	Perkin-Elmer	Estimation of metal

3.4. Microorganism

The strains of *Pseudomonas putida* MTCC(1194) and *Escherichia coli* NCIM(5051) were obtained from MTCC Chandigarh and NCIM Pune respectively.in the form of slant and was subcultured from time to time for its mentainance. The microorganisms were maintained on the medium containing: Beef extract 1.0 g/L, Yeast extract 2.0 g/L, Peptone 5.0 g/L, NaCl 5.0 g/L and Agar 15.0 g/L at pH 7. The medium was autoclaved at 121^0C and 15 lb pressure for 15 min. The media was then poured in the Petri-plates and allowed the agar to solidify. A loop full of microbial colonies were taken from the master slant and inoculated in the Petri plates. The Petri plates were then kept in the incubator. The growth of the bacterial colonies

was observed after 24 hr of incubation at 30^0C. Then the slants were stored at 4°c till further use.

3.5. Inoculum preparation

The experimental studies were carried out in the shake flask as lab scale and in fixed bed bioreactor at large scale under pertinent process variables. The different salts for minimal salt media dissolved in 1000 mL of distilled water. Glucose and peptone were used as primary carbon and nitrogen sources. For degradation study different concentrations of phenol was added as a carbon source and Potassium dichromate ($K_2Cr_2O_7$) was used as the Cr(VI) source.

3.6. Study in shake flask

Degradation capacity of microorganisms was checked individually. i.e phenol degradation capacity of *Pseudomonas putida* (MTCC 1194) and chromium using *Escherichia coli* (NCIM 5051). Phenol degradation was studied at different higher concentrations of phenol ranging from (10 – 1000) ppm by *Pseudomonas putida*. And also Cr(VI) degradation was studied at different concentrations of Cr(VI) ranging from (5-40) ppm by *Escherichia coli*.
Optimizing carbon source and nitrogen source was studied for both the microorganisms.
Optimization of process parameters such as pH, temperature and innoculum volume and age of inoculum were also determined for both the microorganisms.
Simultaneous phenol degradation and Cr(VI) reduction was investigated in the coculture of *Pseudomonas putida* and *Escherichia coli.*
The experimental studies were carried out in the shake flask as batch reactor under pertinent process variables. 100 mL of batch volume was taken along with different concentration of phenol in each of the experiment in which 5% of overnight cultured cells were inoculated and kept in temperature controlled orbital shaker at 30°C in 130 rpm at pH 7 for different time intervals.

The 5% microorganisms were taken from an overnight acclimatized culture were transferred to the liquid media containing different concentrations of phenol and chromium for individual degradation study and for both phenol and Cr(VI) degradation using mixed culture of microorganisms. This Acclimatization was done for both of the microorganisms to

acclimatize the individual microbes to phenol and Cr(VI). In the phenol acclimatization procedure very less amount of glucose with phenol was added to the media for the initial growth of *P. putida*. So *P. putida* started consuming phenol as the sole source of carbon as soon as the glucose was depleted, by this method microbes were made more adaptable to phenol. Also for Cr(VI) acclimatization procedure the *E. coli* was acclimatized to the different concentrations of Cr(VI) to make it more adaptable to efficient utilization of Cr(VI). Phenol-degrading and chromium reducing bacteria are required to be adapted to the phenol and chromium environment. During acclimatization process certain enzymes in the bacteria are induced so that they are available for taking part in the metabolism reaction. This is much more important when dealing with toxic compounds such as phenol and chromium at high concentrations.

The degradation rate is dependent on the nutrients in which the glucose played a major role. It is used by the phenol degrading microorganism for their initial growth and slowly they started consuming phenol. For mixed culture degradation study phenol is used as a sole source of carbon, energy, metabolites and electron donor for chromium reduction by Chromium reducing organism. The added minerals also influenced the growth of organism and enhanced the degradation.

The biomass concentration was observed in a spectrophotometer by using O.D at 600nm. The rpm and temperature were maintained at 130 rpm and 30°C respectively for each set of experiments. The degradation was observed at regular interval of time for both phenol and Cr(VI) degradation and each time the pH was adjusted to 7 for optimum growth of the microorganisms and also for better degradation. The degradation of phenol was studied at 510 nm in a spectrophotometer by knowing the phenol concentration using 4-Amino Antipyrine method at different lengths of time viz. (6,12,18,24,30,36,48,60,66,72,78) hours. The degradation of Cr(VI) was studied at 540 nm in a spectrophotometer by knowing the Cr(VI) concentration using Diphenyl carbazide assay at different lengths of time viz. (3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48) hours. Each concentrations of phenol and Cr(VI) level for both of the microbes were repeated twice or thrice to get the best result. Data for the phenol degradation were obtained at regular 6 hour interval of time and the data for the Cr(VI) degradation were obtained at regular 3 hour interval of time. And pH was adjusted to 7 at regular interval of time. Also the data for biomass concentration in terms of OD were obtained at regular 3 hour interval of time. Both phenol and Cr(VI) degradation was done by

taking different initial concentrations of both the pollutants to study the inhibition effect of both the pollutants on each other.

3.7. Study in bioreactor

Large scale simultaneous degradation of phenol and reduction of Cr(VI) using mixed culture of microorganisms was carried out in a bioreactor. Degradation of aqueous solution of phenol with chromium and textile industry effluent was performed at its optimum conditions in a bioreactor (New Brunswick, BIO FLOW 410) using the microbial strains of phenol degrading bacteria *Pseudomonas putida* (MTCC 1194) and chromium reducing bacteria *Escherichia coli* (NCIM 5051). The bioreactor is having 3 liters working volume with auto sterilization option and all process parameters in the bioreactor like temperature, pH, DO and addition of anti foam was controlled automatically. The optimum conditions like pH 7, temperature 30^0C, agitation 200 rpm, DO 100 ppm were automatically maintained throughout the study in the bioreactor into which 12 hour old 5% inoculum volume were added. Sampling was done regular interval of time to study the degradation kinetics of both phenol and chromium and the biomass formation. The results of the treatment of textile industry waste water indicates that all water parameters including the concentration of phenol and chromium along with other heavy metals is reduced and brought to their permissible limits.

3.8. Media optimization

The Biomass growth studies along with maximum phenol and chromium degradation were carried out using various organic and inorganic nitrogen sources (Ammonium chloride, Yeast extract and Peptone) and various carbon sources (Dextrose, Starch soluble and Sucrose). For the study 100 ppm phenol and 10 ppm Cr(VI) was used. The optimized parameters are further considered for degradation of both phenol and chromium in shake flask and bioreactor also for both aqueous and industrial effluent.

3.9. pH optimization study

The effect of pH on chromium reduction and phenol degradation was also studied by taking three pH variables i.e pH 6, 7 and 8. The respective pH was adjusted with 1N NaOH and 1N HCl.

3.10. Temperature optimization study

The effect of temperature on phenol degradation was also studied under three temperature variables i.e 25^0C, 30^0C and 35^0C. The study was carried out in the temperature controlled shaking incubator.

3.11. Optimization of inoculum volume

The effect of inoculum volume on chromium reduction and phenol degradation was also studied by taking four volume of inoculum variables i.e 1%, 3%, 5% and 7%.

3.12. Analysis under 100X microscope

Changes in the cells of mixed culture of microorganisms occurred before and after degradation and the formation of metabolites were observed under 100X microscope.

3.13. SEM, EDX and AAS analysis

Scanning Electron Microscopy-Energy Dispersive X-ray Spectroscopy analysis (SEM-EDX) is evaluated to understand the cell morphology, elemental composition of both the untreated and treated industrial effluent. The SEM-EDX analyses were carried out with the help of a computer controlled field emission SEM equipped with EDX detection system. Detailed examination by SEM helped us to detect the morphological and topological changes that occurred in the microbial cells before and after degradation. Similarly the industrial effluent was also examined using EDX for the conformation of the presence of pollutants and the heavy metals before and after treatment. Also the amount of other heavy metals presence in the textile effluent before and after treatment were analysed with the help of Atomic Absorption Spectroscopy.

3.14. Total chromium analysis

Total chromium present in the media was estimated by the Atomic Absorption Spectroscopy (AAS). The Atomic Absorption Spectroscopy reading showed the amount of Cr(VI) reduces gradually to Cr(III) and the chromium which is completely degraded.

3.15. Phenol estimation by 4-Amino Antipyrine method

- The phenol concentration in the sample was determined using the 4-Amino Antipyrine method. The reaction mixture containing 0.9mL distilled water, 0.1mL sample, 50µL of 2N Ammonium hydroxide, 25µL of 2% 4-Amino Antipyrine and 8% $K_3Fe(CN)_6$ was added after mixing at each step. Then it was centrifuged at 14,000 rpm for 2 minutes. Then the supernatant is collected and read the absorbance in spectrophotometer at 510 nm against reference that except phenolic material.

- In this method phenolic material reacts with 4-Amino Antipyrine in the presence of K_3 $Fe(CN)_6$ at a pH of 10 to produce a purple-red coloured end product whose absorbance is to be checked in the spectrophotometer at 510 nm.

3.16. Diphenyl carbazide assay for Cr(VI) reduction

- Transfer 9.5 mL of the extract to be tested to a 10 mL volumetric flask. Add 200µL Diphenylcarbazide solution and mix. Add H_2SO_4 solution to give a pH of 2 +/- 0.5, dilute to 10 mL with reagent water, and let it stand for 5 to 10 min for full color development. Transfer an appropriate portion of the solution to a 1cm absorption cell and measure its absorbance at 540 nm. Use reagent water as a reference.
- In this method the hexavalent chromium reacts with the 1-5, Diphenylcarbazide at a pH 2 +/- 0.5 to form a red-violet coloured end product whose absorbance is checked in the spectrophotometer at 540 nm by comparing with standards which are expressed in ppm [Method 7196A].

3.17. Calibration plot for phenol and chromium

Calibration plot was drawn by taking the known phenol and chromium concentrations and their absorbance. Absorbances of different known phenol and chromium concentrations (1, 2, 3, 4 and 5) ppm were studied by spectrophotometer at 510nm and 540 nm respectively using the procedure mentioned earlier. After plotting absorbance vs concentration a linear graph was obtained.

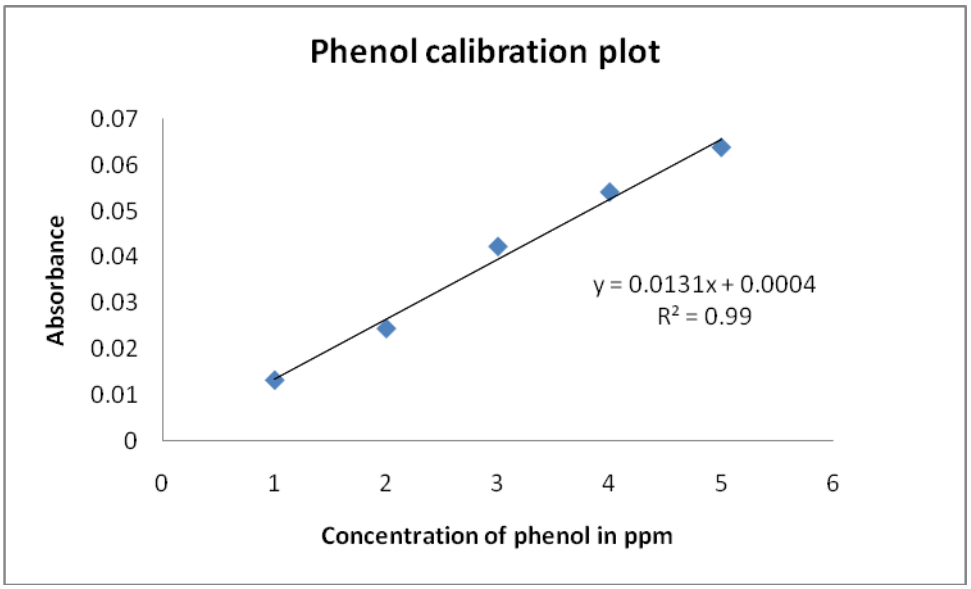

Fig. 1: Calibration plot for phenol

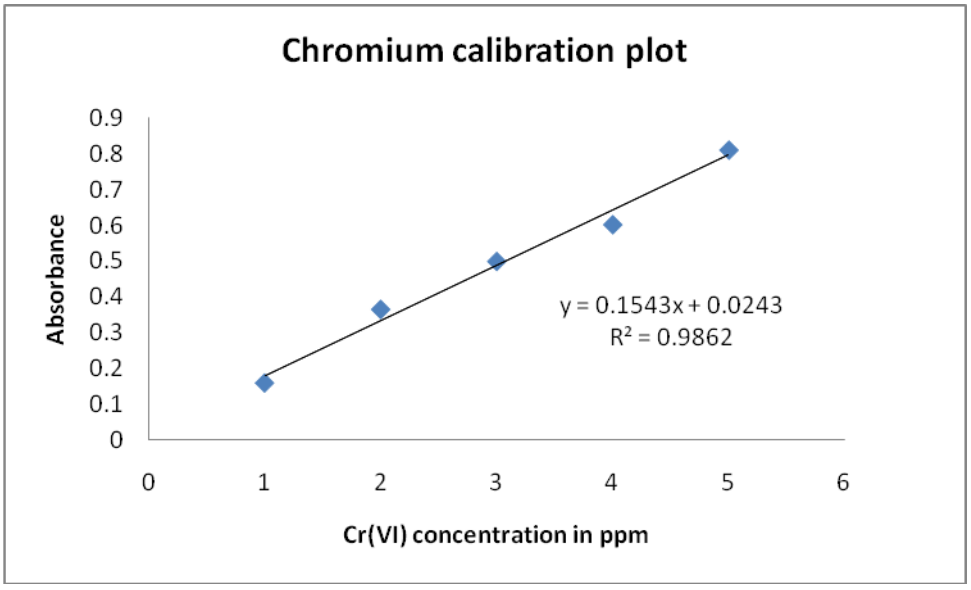

Fig. 2: Calibration plot for Cr(VI)

The R^2 values suggested that the plots above are accurate (fig.1 and fig.2). The concentration of unknown phenol sample can be obtained by measuring its absorbance at 510nm. Similarly the concentration of unknown chromium sample can be obtained by measuring its absorbance at 540nm. The corresponding concentration value from the above plot will estimate actual concentration of the unknown sample.

3.18. Biomass study

1 ml culture was taken in a 1.5 ml centrifuge tube and allowed to centrifuge at 10,000 rpm for 5 minutes. The supernatant is discarded and the pellet is washed by taking 1 ml distilled water. The pellets were washed with distilled water several times and the optical density of bacterial cell suspension was determined by turbid metric measurement in a spectrophotometer at 600 nm by taking distilled water as blank.

3.19. Resistant to other heavy metals

Maximum tolerance level of other metals by the mixed culture of microorganisms present in the medium along with phenol and chromium were examined. Mixed culture of the organisms were allowed to grow on agar plate containing heavy metals Zn, Cu, Ni each present at a concentration of 10mg/L. Number of colonies were counted after incubation of 48 hrs with both the microorganisms.

4. RESULT AND DISCUSSION

4.1. Physico-chemical characteristic of industrial effluent

The industrial effluent was collected from the local textile industry and the physico-chemical parameters were estimated using standard methods. The physicochemical parameters of the Industrial Effluent estimated are shown in Table 2. The effluent had a reddish brown colour. The pH of the effluent were measured using an ion specific pH electrode and were found to be in the range of (7.9-8.2). This indicated that the effluent from the textile industry is slightly alkaline in nature. High degree of contaminants in the water generally induces unfavourable physiological reactions in its consumers. Thus Analysis of the solids is important parameter in the biological wastewater treatment process. The Total Dissolved solids (TDS) and Total suspended solids (TSS) in the effluent were measured and found to be in the range of (2580-2670) ppm and (280-3300) ppm respectively. Similar results reported by other researchers [Badani et al., (2005); Eswaramoorthi et al., (2007); Hai et al., (2003)] treatment of textile industry effluent. Other important parameters like COD and BOD were also estimated. The COD of water is generally 2 to 3 times than that of BOD (Greg and Sewer). Similarly in our study the COD and BOD were found to be in the range of (410-480) mgL^{-1} and (1120-1170) mgL^{-1} respectively. The effluent was also subjected to atomic absorption spectroscopy to analyse the exact amount of the pollutants present. In this study amount of copper varied from (6–8) mgL^{-1}, Zinc (9-11) mgL^{-1} and Nickel in the range of (5-8) mgL^{-1} respectively. The analysis of two major pollutants like phenol and chromium were done using 4-Amino Antipyrine assay and 1-5 Diphenyl carbazide assay and were found to be in the range of (240-250) ppm and (3-5) ppm respectively.

Table 2: Physiochemical Characterization of Textile industry Effluent collected from Rajahmundry, AP, India.

Parameters	**Effluent (Untreated)**
Colour	Reddish Brown
pH	7.9- 8.2
BOD (mg/l)	410-480
COD (mg/l)	1120 -1170
TOC (mg/l)	207
TDS (mg/l)	2580-2670
TSS (mg/l)	280-330
PHENOL (mg/l)	250
Cr(VI) (mg/l)	5
TOTAL Cr (mg/l)	5
Zn (mg/l)	9-11
Cu (mg/l)	6-8
Ni (mg/l)	5-8

Chemical analysis of a typical textile industry wastewater indicated that it contained other major contaminants, including sulphates and heavy metal ions such as iron, aluminium and zinc, copper, nickel, chromium and organic pollutants like phenol. Similar pollutants were reported by researchers [El-Naas et al., (2009); Nosheen et al., (2000); Ali et al., (2009)].

4.2. EDX analysis of the textile industry effluent

Energy dispersive X-ray spectroscopy (EDX) is evaluated to understand the elemental composition of the textile industry effluent. The graph obtained from the analysis of the effluent with EDX shows the presence of various pollutants like Cr, Zn, Ni and Cu. Our result conforms to the finding of other researchers [Srivastava and Thakur, (2007); Francisco et al., (2010); Li et al., (2008); Das and Mishra, (2009)].

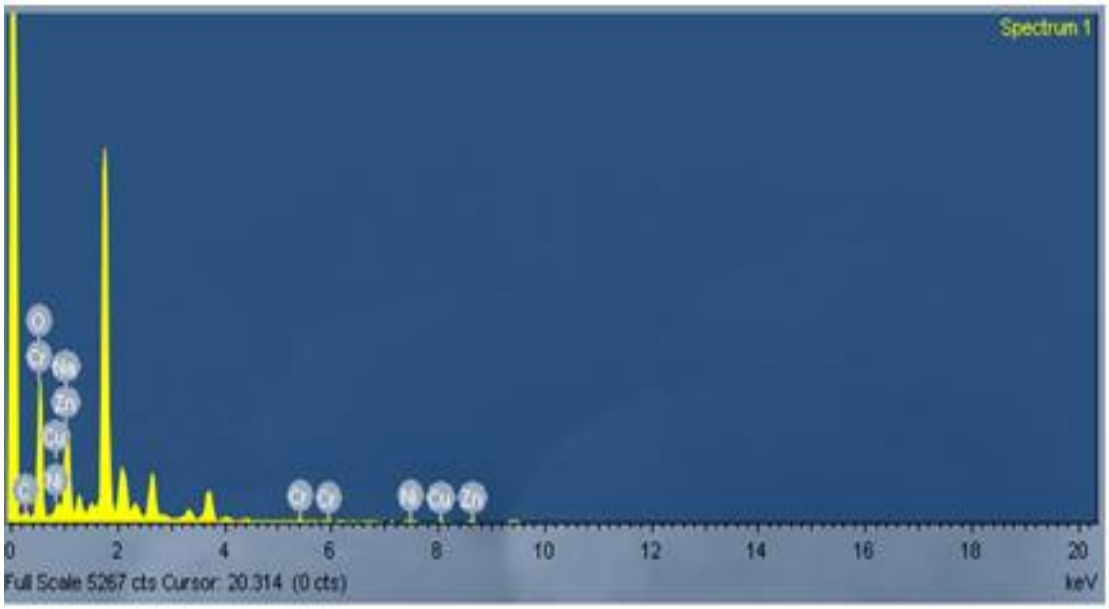

Fig. 3: EDX of untreated textile industry effluent

4.3. Microorganism

The strains of *Pseudomonas putida* MTCC(1194) and *Escherichia coli* NCIM(5051) were obtained from MTCC Chandigarh and NCIM Pune respectively in the form of slant and was sub cultured from time to time for its maintenance. The Petri plates were maintained in a incubator at 30^0C and the slants were stored at 4°C till further use.

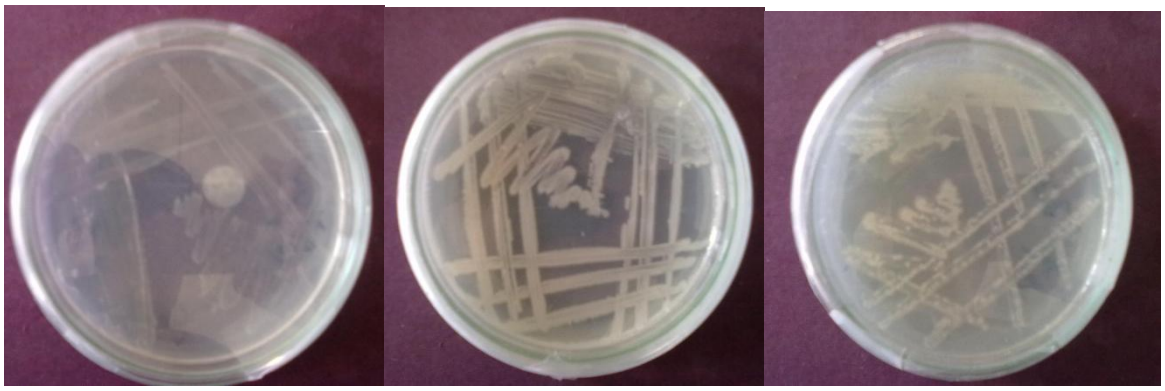

Fig. 4: Image of *P. putida* on agar plate

Fig . 5 : Image of *E .coli* on agar plate

Fig 6. : Mixed culture of *P. putida* and *E. coli* on agar plate

4.4. Media optimization study

The Biomass growth studies along with maximum Phenol and Chromium degradation were carried out using various organic and inorganic nitrogen sources (Ammonium chloride, Yeast extract and Peptone), various carbon sources (Dextrose, Starch soluble and Sucrose) and a

initial Phenol and Chromium levels of 100 ppm phenol and 10 ppm respectively. Maximum degradation of phenol and chromium along with optimum growth of biomass of both the organisms were monitored under various media components. The biomass obtained by both in the presence of organic nitrogen sources was found to be greater than the inorganic nitrogen sources. The highest biomass productivity along with better degradation of phenol by *P. putida* and Cr(VI) reduction by *E. coli* was obtained when peptone was used as a nitrogen source in the media followed by Yeast extract, and Ammonium chloride (Fig. 7 and Fig. 9). The highest biomass productivity along with maximum phenol and Cr(VI) degradation was obtained by both the strains when Dextrose was used as a carbon source in the media (Fig. 8 and Fig. 10). It is inferred that Dextrose being monosaccharide was easily degraded by the organisms. In a Similar study by Bae et al., (2000) and Shen and Wang, (1994) the maximum reduction of Cr(VI) to a less toxic trivalent chromium by *E. Coli* ATCC 33456 also evidenced in the presence of dextrose. Thus during the media optimization study *E. coli* completely degraded 10 ppm of Cr(VI) in (12-15) hours with a high biomass O.D of 3.03 with peptone as nitrogen source and a biomass O.D of 2.88 with dextrose as a carbon source in 24 hours as shown in (Fig. 7and Fig. 8).

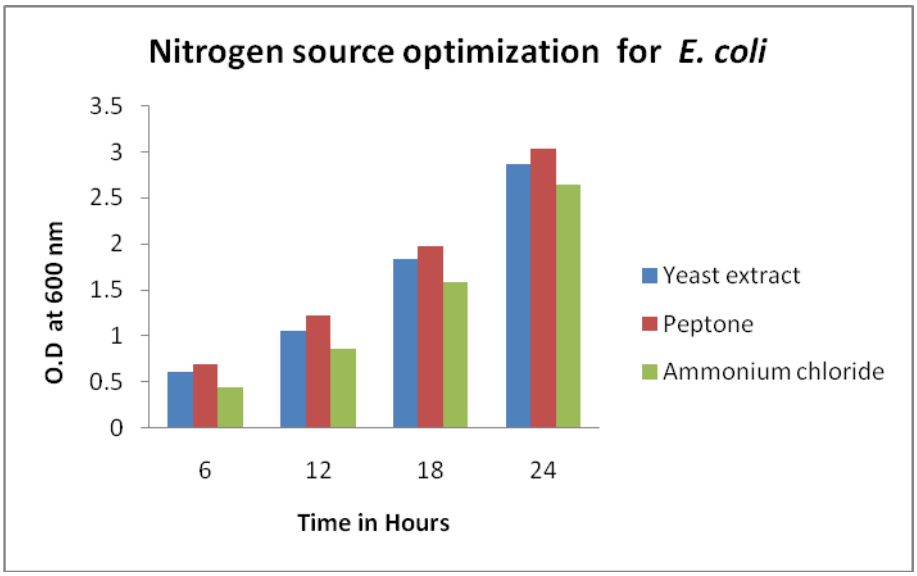

Fig. 7: Biomass growth of *E. coli* at various nitrogen sources with 10 ppm Cr(VI)

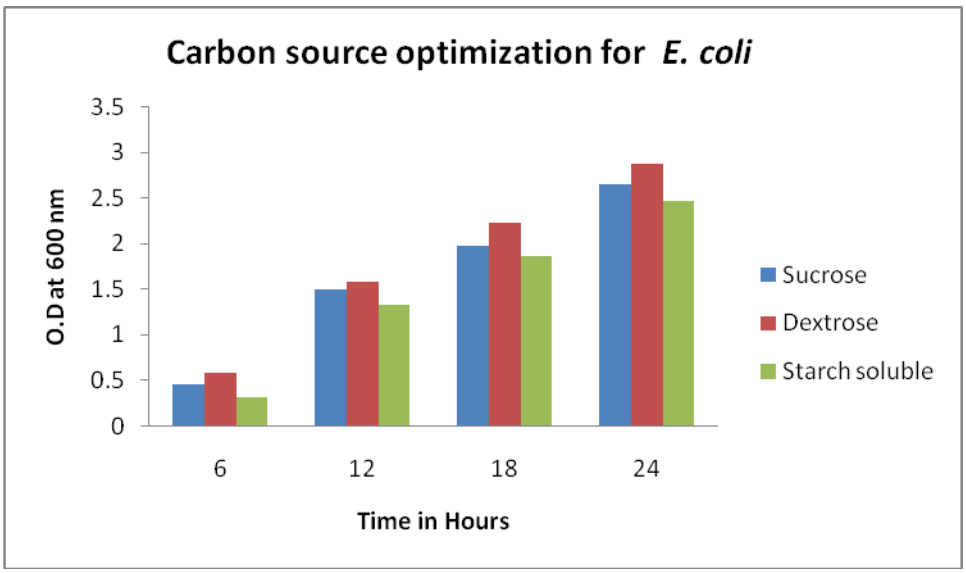

Fig. 8: Biomass growth of *E. coli* at various carbon sources with 10 ppm Cr(VI)

Other studies reported by Ramakrishna and Philip, (2005) and Das and Mishra, (2009) suggest the effect of different electron donors on Cr(VI) reduction using *Ganoderm lucidum* and *Brevibacterium casei* respectively. Among several electron donors and nitrogen sources screened, dextrose was the optimum electron donor and peptone showed maximum Cr(VI) reduction followed by yeast extract and inorganic nitrogen sources.

Similarly Khazi et al., (2007), reported that glucose played a major role in phenol degradation and was used by the *P. putida* MTCC 1194 initially as simple source of carbon for its mass multiplication and growth. The microbes then slowly started consuming phenol as a carbon source for their growth. Kotresha and Vidyasagar, (2008) achieved 100% phenol degradation using *P. aeruginosa* MTCC 4996 with peptone and dextrose as nitrogen and carbon source respectively. During the media optimization study *P. putida* completely degraded 100 ppm phenol in (12–15) hours with a high biomass O.D of 2.68 when peptone was used as a nitrogen source and a biomass O.D of 2.56 with dextrose as a carbon source in 24 hours as shown in (Fig. 9 and Fig. 10).

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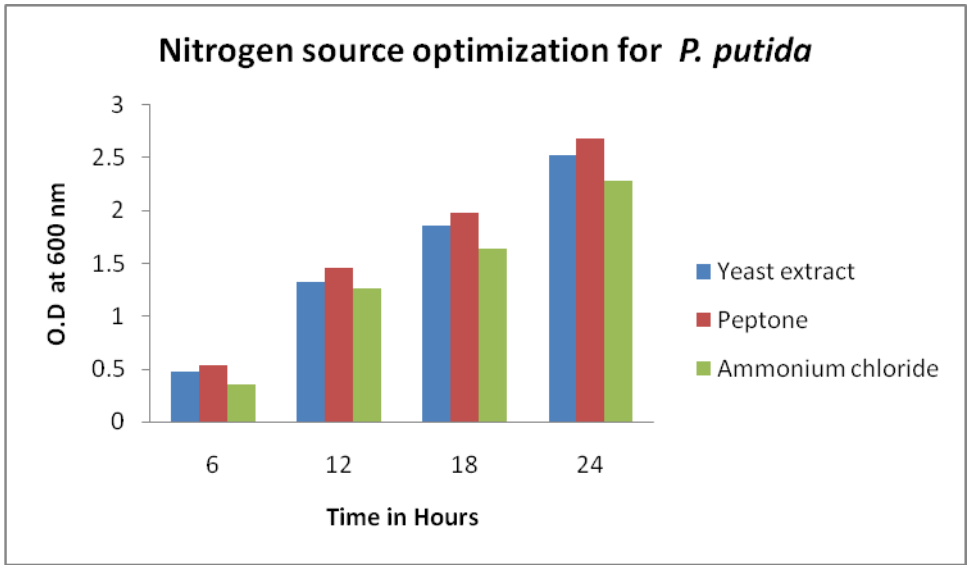

Fig. 9: Biomass growth of *P. putida* at various nitrogen sources with 100 ppm phenol

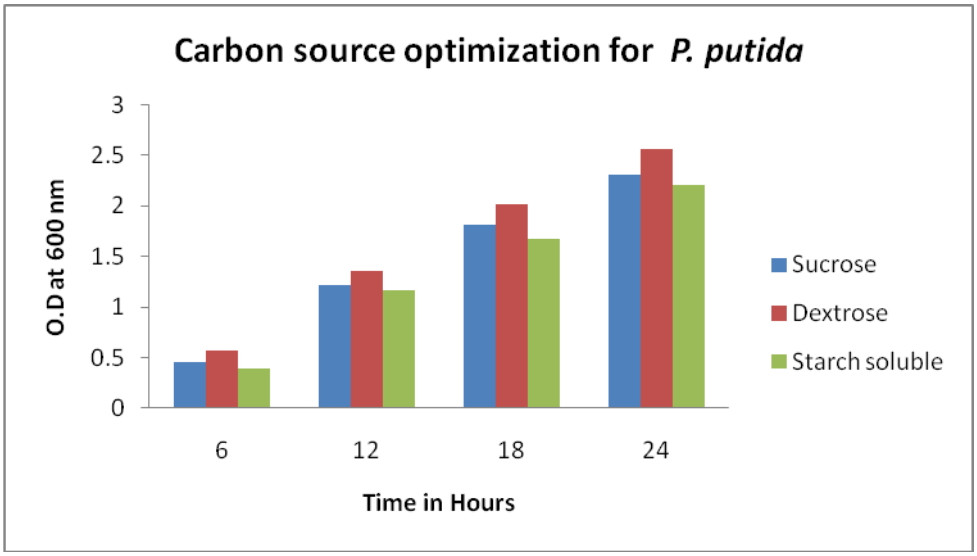

Fig. 10: Biomass growth of *P. putida* at various carbon sources with 100 ppm phenol

Our results were contradictory to Annadurai et al., (2002) who reported microbiological degradation of phenol using mixed liquors of *Pseudomonas putida* and activated sludge and ammonium sulphate as the source of nitrogen. Similarly Suhaila et al., (2010) reported efficient degradation of phenol and growth of *Rhodococcus* UKM-P using Ammonium sulphate as the nitrogen source. These observations suggest that optimum degradation characteristics of each organism are influenced by different nitrogen and carbon source.

4.5. pH optimization study

4.5.1. Effect of pH on Chromium reduction

Fig. 11.a shows the effect of pH on Chromium reduction by *E. coli*. The study was carried for the reduction of 15 ppm chromium at varying pH 6, 7 and 8. Freshly prepared overnight culture of *E. coli* was inoculated with 15 mg/L of Cr(VI) and maintained at a incubation temperature of 30^0C and at a shaking speed of 130 rpm. pH was maintained throughout the experiment using 1N NaOH and 1N HCl.

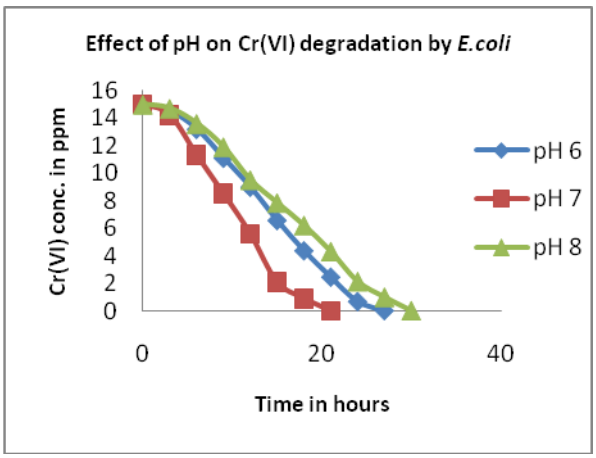

Figure 11.a

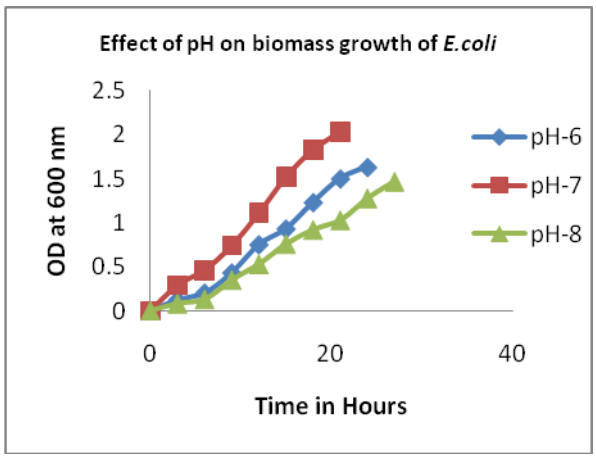

Figure 21.b

Fig. 11.a: Effect of pH on Cr(VI) degradation by *E. coli* at Cr(VI) concentration of 15 ppm

Fig. 11.b: Effect of pH on biomass growth of *E. coli* at Cr(VI) concentration of 15 ppm

From the results shown in Fig. 11(a&b), pH was found to greatly influence the biomass growth as well as Cr(VI) degradation. Under extreme pH conditions (6 and 8) the bacterial growth was found to be reduced and also the rate of Cr(VI) reduction decreased. pH 7 on the other hand not only gives a high biomass growth of *E. coli* but also higher degradation of chromium. Thus at an optimum pH of 7, *E. coli* efficiently degraded 100% Cr(VI) in a short period of (20-22) hours as compared to pH 6 and 8 where prolonged degradation was observed. Though Cr(VI) reduction is an enzymatic process variation in pH will affect the enzyme activity [Farrell and Ranallo, (2000)]. Similarly Bae et al., (2000) and Shen and Wang, (1994) evaluated the optimum pH for Cr(VI) reduction by *E. coli* ATCC 33456 within a pH range of (3-8) reported that the optimum pH for better chromium reduction was 7.0. Das and Mishra, (2009) studied the degradation of chromium within a pH range of (4-9)

and optimum result reported at pH 7 using *Brevibacterium casei*. Poornima et al., (2010) also reported maximum chromium reduction at pH 7.

4.5.2. Effect of pH on phenol degradation

Fig. 12.a shows the effect of pH on phenol degradation by the bacterial strain *P. putida*. The study of phenol degradation by *P. Putida* at 500 ppm initial concentration was carried out at varying pH 6, 7 and 8. Freshly prepared overnight acclimatized culture of *P. Putida* were inoculated with 500ppm of phenol and maintained at an incubation temperature of 30^0C and 130 rpm of shaking. pH was maintained throughout the experiment using 1N NaOH and 1N HCl.

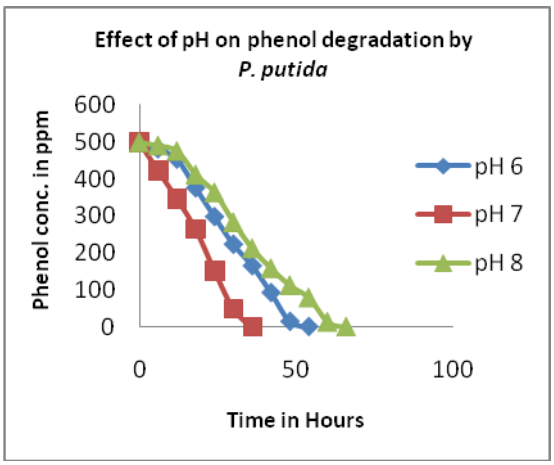

Figure 32.a

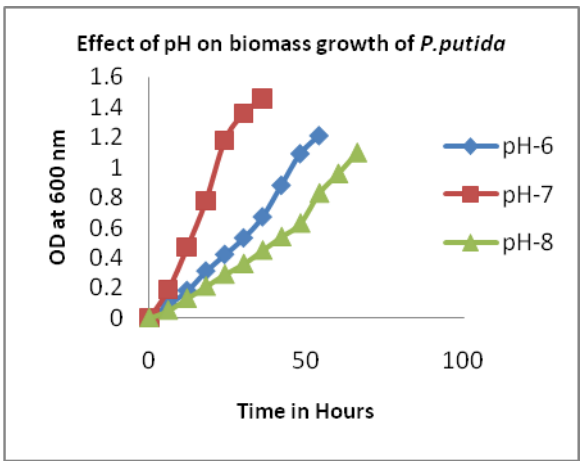

Figure 42.b

Fig. 12.a: Effect of pH on phenol degradation by *P. putida* at phenol concentration of 500 ppm

Fig. 12.b: Effect of pH on biomass growth of *P. putida* at phenol concentration of 500 ppm

As shown in Fig. 12(a&b) above phenol degradation was highly influenced by the variation in pH. At an optimum pH of 7 *P. putida* was able to degrade 500 ppm of phenol in less time i.e (34-36) hours as compared to pH 6 and 8 which took (52-54) hours and (64-66) hours respectively. In a similar work by khazi et al in 2007, different ranges of pH (4, 5, 6, 7 and 8) were chosen for the study and it was found that initial pH had a great influence on the growth and degradation rate. Among the various pH values, pH 7 was found to produce more amounts of cell mass and resulted in better degradation of phenol. Bandyopadhyay et al., (1998) studied the kinetics of phenol degradation by *P. putida* MTCC 1194 and found that at pH value of 7 the degradation was optimum. Similar results were reported by Agarry et al.,

(2009) in which maximum phenol degradation was observed using indigenous *Pseudomonas fluorescence*. In a similar study Zeng et al., (2010) reported two new isolate of high phenol-degrading strains, *Micrococcus* sp. and *Alcaligenes faecalis* JH 1013, gave highest degradation and growth at pH 7.0. Similarly, Veenagayathri and Vasudevan, (2010) studied the degradation of 100 mg/L phenol under the effect of different pH ranging from (5.5 to 8.5) by the bacterial consortium. They reported maximum phenol degradation with 99% efficiency at pH 7. They also observed degradation of (5%, 9%, 24%, 42%, 74%) at different pH (5.5, 6, 6.5, 8.5 and 8) respectively.

4.6. Temperature optimization study

The incubation temperature greatly influences the metabolic activity of the microbe and the degradation of Cr(VI) or phenol. The effect of temperature on phenol and chromium degradation was studied at a temperature range of 25^0C to 35^0C. Effect of temperature on phenol and chromium reduction using overnight grown cultures was studied at pH 7, initial phenol and chromium concentration of 500 ppm and 15 ppm respectively.

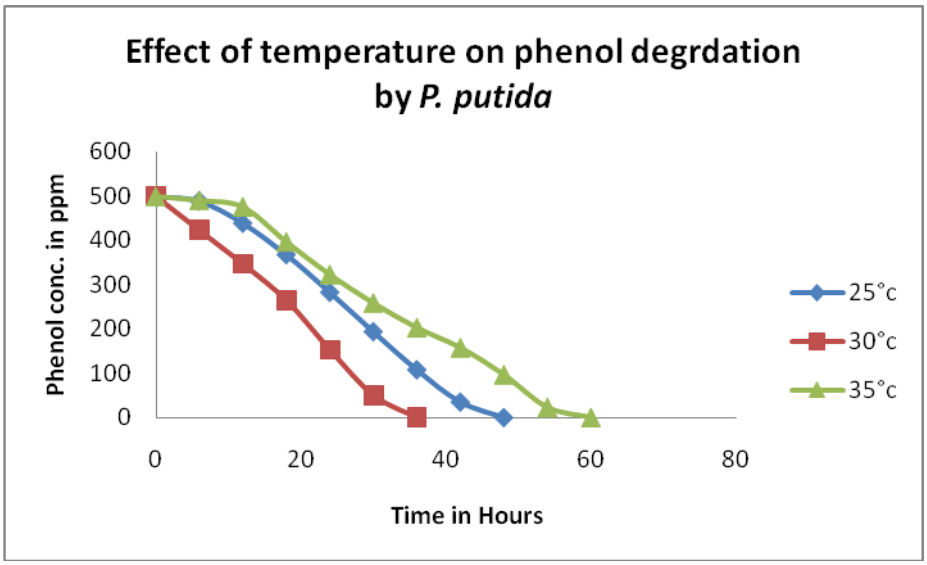

Fig. 13: Effect of temperature on phenol degradation by *P. putida* at phenol concentration of 500 ppm

As shown in fig. 13 at 30°C *P. putida* was able to degrade 500 ppm of phenol early in (34-36) hours as compared to 25°C and 35°C. At 25°C *P. putida* degraded 500 ppm of phenol in (46 – 48) hours whereas the degradation was slowest at 35 °C where complete degradation was only achieved after 60 hrs of incubation time. The probable reason behind this might be the

increase in lag phase at higher temperature condition of 35°C. Similarly Hank et al., (2010) reported the effect of temperature on the degradation of phenol by *Pseudomonas aeruginosa* at 30°C and 40°C under similar operational conditions. Degradation was best at 30°C with a total degradation of phenol in 100 hours. At 40°C the time of the degradation was prolonged to 160 hours. From the literature it is evident that Cr(VI) degradation by *E. coli* is optimum at 37°C. But in our study the degradation of both phenol and Cr(VI) in the presence of coculture of *P. putida* and *E. coli* is to be monitored. In our study efficient chromium reduction was observed at a temperature of 30°C by *E. coli* (NCIM 5051). The phenol degradation by *P. putida* is optimum only at 30°C beyond that temperature it didnot show better degradation. So it can only be possible to maintain the temperature condition at 30°C. Chakraborty et al., (2010) isolated phenol degrading bacteria from coke processing unit and obtained optimum phenol degradation at 30°C. Similarly Suhaila et al., (2010) studied the phenol degradation by using *Rhodococcus* UKM-P in shake flask culture under optimum process conditions. About 500 mg/L phenol was degraded after 21 hours of cultivation at an optimum temperature of 30^0C.

4.7. Optimization of inoculum age and volume

4.7.1. Effect of inoculum age on phenol and Cr(VI) degradation

Phenol degradation and chromium reduction in the shake flask culture were studied for different lengths of time like (6, 12, 18 and 24) hours, The cells of *P. putida* and *E. coli* were grown in a tertiary culture of mineral salt medium for different lengths of time from which inoculums were collected and tested for both phenol degradation and chromium reduction. The inoculum of 12 hours age demonstrated the highest rate of phenol degradation and chromium reduction for both the cultures.

4.7.2. Effect of inoculums volume on phenol degradation and Cr(VI) reduction

The volume of inoculums was found to have a great impact on the degradation of phenol and reduction of chromium as shown in fig. 14 and fig. 15 respectively. The effect of volume of inoculum was studied using different volumes (1%, 3%, 5% and 7%) of the 12 hours grown culture with 5 ppm and 250 ppm of Chromium and Phenol respectively.

From the experimental results it is evident that *E. coli* completely degrades 5 ppm of Cr(VI) in (10-12) hours when 5% inoculum volume was used as compared to 1% and 3%.Similarly in the case of phenol degradation it was found that *P. putida* takes around (18-21) hours to completely degrade 250 ppm of phenol when 5% inoculum volume was used. 1% and 3% volume inoculums on the other hand took longer time for complete degradation. Thus our result showed the most suitable inoculum age and volume for highest phenol degradation by *P. putida* as 12 hours and 5% inoculum. Our result also showed similarity with those of Bae et al., (2000) for Cr(VI) reduction, Bandyopadhyay et al., (1998) and Khazi et al., (2007) for phenol degradation. Similarly *E. coli* ATCC 33456 showed optimum chromium degradation when 5% inoculum volume of 12 hours old culture was used for the study [Bae et al., (2000)]. These may be explained by the fact that increase of inoculum concentration perhaps reduces the lag time and later becomes least at the inoculum concentration of 7% v/v. However inoculum volume 5% and 7% showed very little changes in degradation behaviour, hence 5% inoculum volume was selected for further study.

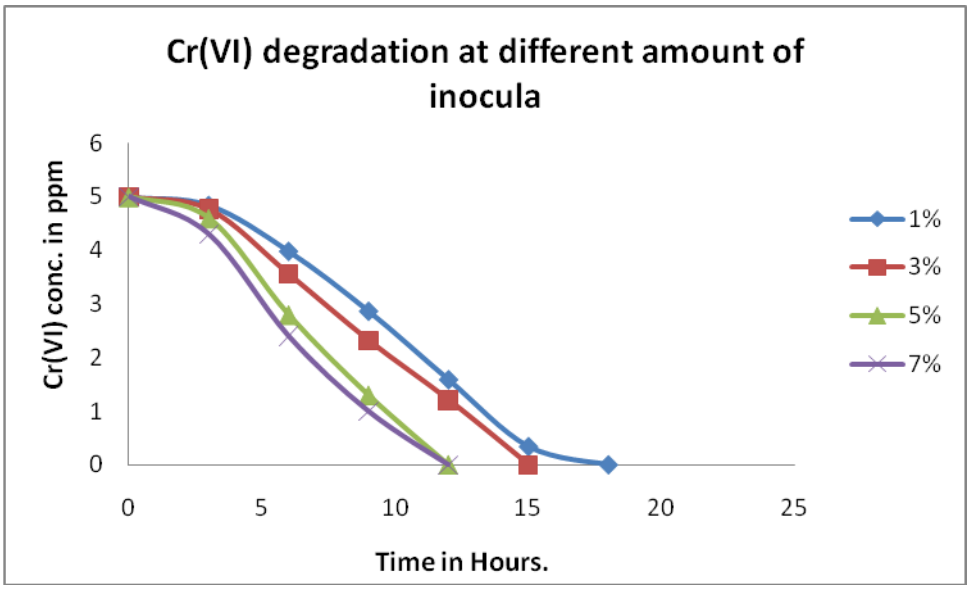

Fig. 14: Effect of inoculum volume on Cr(VI) degradation by *E. coli at* Cr(VI) concentration of 5 ppm

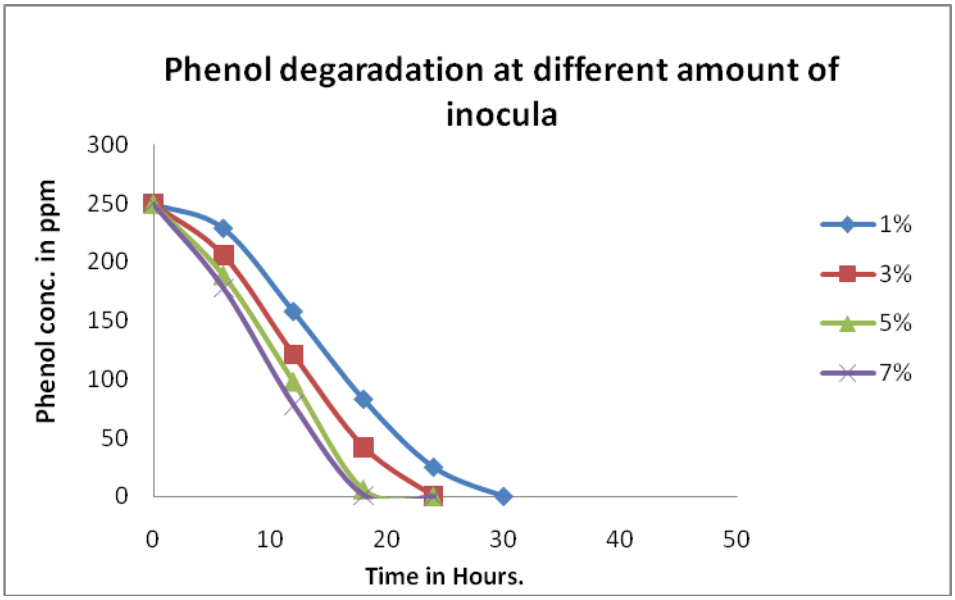

Fig. 15: Effect of inoculum volume on degradation by *P. putida* at phenol concentration of 250 ppm

4.8. Chromium degradation study using Escherichia coli

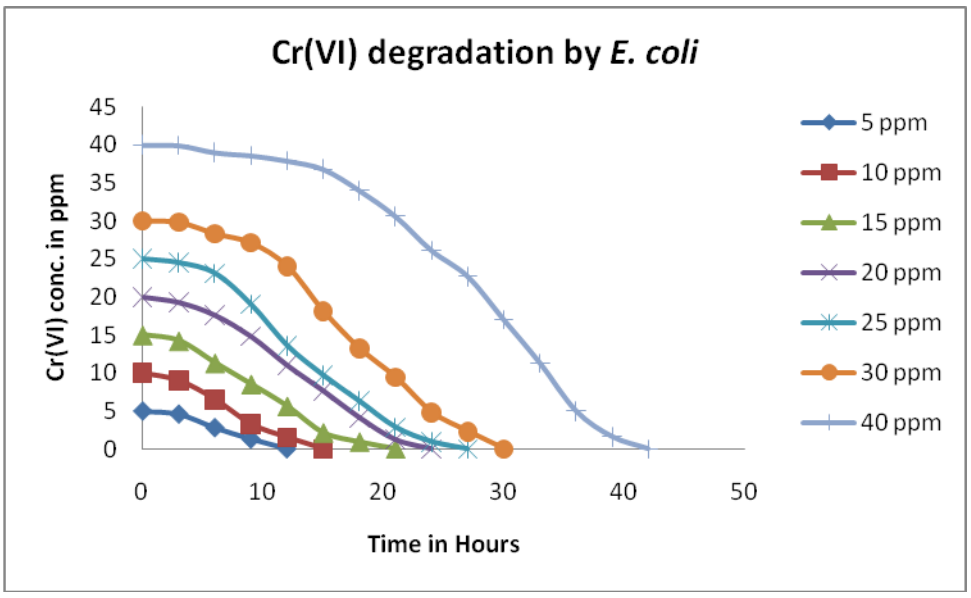

Fig. 16: Degradation of different concentration of Cr(VI) by *E. coli* at optimum conditions: temperature 30^0C, pH 7, 12 hours old 5% inoculum volume, shaking at 130rpm.

Individual Chromium degradation using the *Escherichia coli* at various Cr(VI) concentrations ranging from (5,10,15,20,25,30,40) ppm were checked at optimum conditions like temperature 30^0C, pH 7, 5% inoculum volume, shaking at 130 rpm, with peptone and dextrose as nitrogen and carbon source respectively. In the case of optimum conditions required for Cr(VI) reduction in *E. coli* NCIM(5051) shows similar result as Bae et al.,

(2000) done for Cr(VI) degradation by *E. coli* ATCC 33456. They have reported that better Cr(VI) reduction can be done at optimum conditions like temperature 30^0C, pH 7, inoculum volume 5%, overnight grown culture and dextrose used as a electron donor. Under these optimum conditions *E. coli* was able to completely degrade maximum of 40 ppm of Cr(VI). Whereas Cr(VI) degradation by Bae et al., (2000) using *E. coli* ATCC 33456 shows maximum 89.9 % removal. From their study it was found that *E. coli* ATCC 33456 took around 18 hours to completely reduce 5ppm of Cr(VI) where as our culture *E. coli* NCIM (5051) efficiently remove 5 ppm of Cr(VI) in around 12 hours. From the above graph it was observed that the degradation kinetics increased with increasing initial Cr(VI) concentration that suggested that the time required for complete reduction of Cr(VI) at different concentration goes on increasing as the concentration of Cr(VI) goes on increasing. 5 ppm of Cr(VI) takes 12 hours for complete degradation where as 40 ppm of Cr(VI) takes (42–43) hours for its complete degradation. A extended lag phase is clearly observed at higher ppm levels like 30 ppm and 40 ppm of Cr(VI). The growth of microbial population reduces with increasing concentration of Cr(VI). Konopka et al., (1999) confirmed that the microbial biomass generation decreased as the concentration of heavy metal increased. According to Vasanthy, (2004) the growth or production of biomass increased with incubation period and reached the maximum at equilibrium time and then remained constant. He reported that *Bacillus* sp. was effective in Cr(VI) removal up to 83.4% at 10 ppm and 79.1% at 50 ppm concentration after 72 hours of incubation. Parameswari et al., (2009) reported that the time for removal of maximum metal (Cr and Ni) at initial metal concentration of 25 ppm by *A. chroococcum, Bacillus* sp. and *P. fluorescens,* respectively was found to be 72 hours. Megharaj et al., (2003) reported that using *Arthrobacter* sp. maximum 20mg/l Cr (VI) was degraded and 100% degradation was achieved in 45 hours. Also at 50mg/l Cr(VI), 60% degradation was achieved in 70 hours. Using *Bacillus* sp. maximum of 10mg/l Cr(VI) was completely degraded in 70 hours and 20mg/L Cr(VI) achieved 60% degradation after 70 hours.

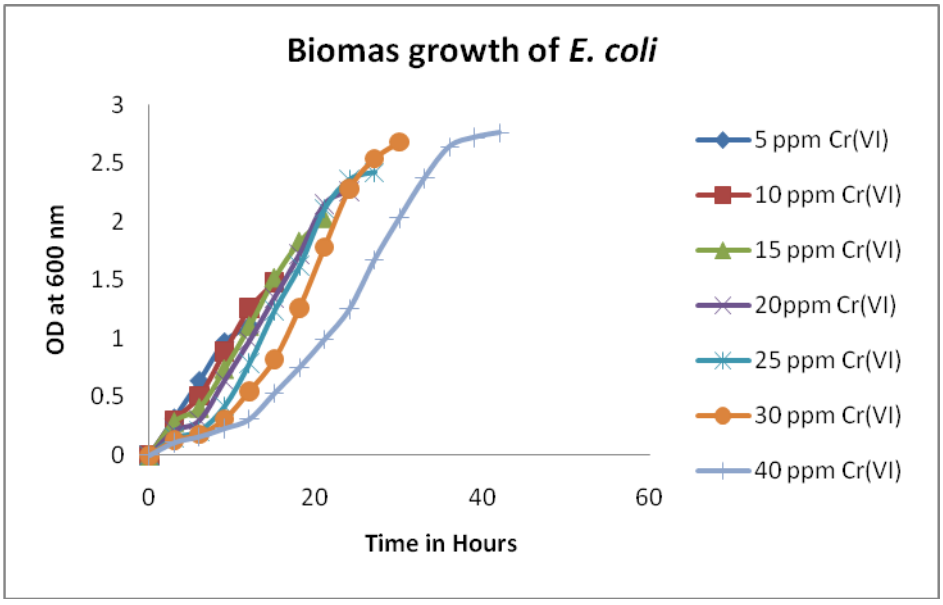

Fig. 17: Effect of initial concentrations of Cr(VI) on Biomass growth of *E. coli* at optimum conditions: temp 30^0C, pH 7, 12 hrs old 5% inoculum volume, shaking at 130 rpm.

Biomass growth of *E. coli* in the form of O.D at 600nm was taken under optimum conditions at different Cr(VI) concentrations in regular time intervals. It shows that higher Cr(VI) concentrations *E. coli* shows extended lag phase. And similar finding were observed by Bae et al., (2000) when *E. coli* ATCC 33456 was used for Cr(VI) degradation. Similarly Das and Mishra, (2010) studied Cr(VI) reduction using *Brevibacterium casei* and found rapid growth phase of the bacterium up to 12 hours after which it remains stationary. In a study carried out by Poornima et al., (2010) using *Pseudomonas putida*; *Pseudomonas plecoglossicida* it was found that the microbe had a extended lag phage and was stationary after 12 hours.

4.9. Phenol degradation study using Pseudomonas putida

Effect of phenol degradation using the *Pseudomonas putida* at various phenol concentrations ranging from (10,50,100,250,500,750 and 1000) ppm were also studied at optimum parameter conditions that is temperature 30^0C, pH 7, 5% inoculum volume, shaking at 130 rpm, peptone and dextrose as nitrogen and carbon source respectively.

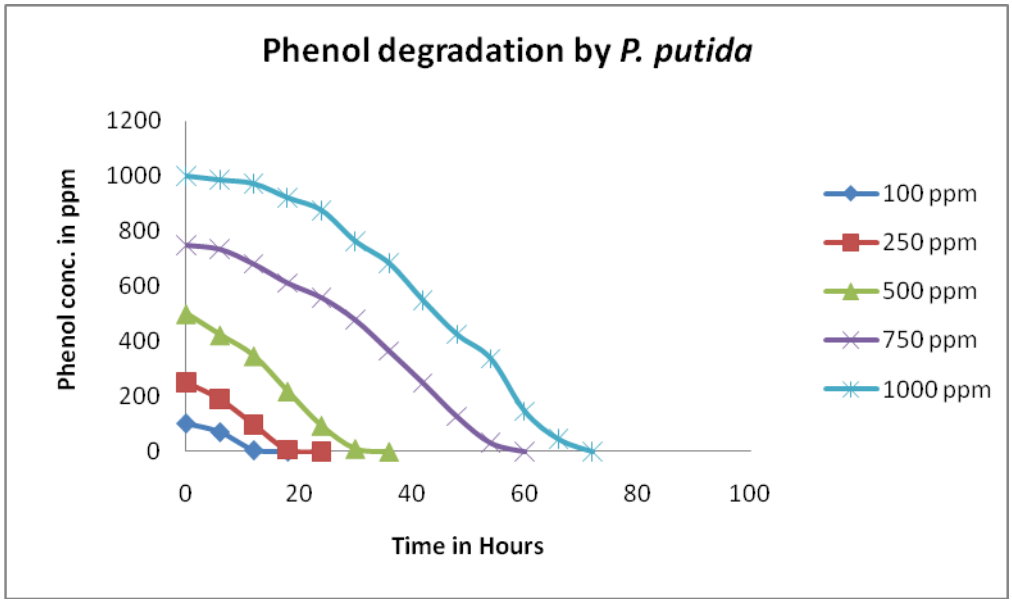

Fig. 18: Degradation of different concentration of phenol by *P. putida* at optimum process conditions: temp 30^0C, pH 7, 12 hours old 5% inoculum volume, shaking at 130 rpm.

The experimental results are shown in fig.18 degradation is highly dependent upon phenol concentration. As the concentration of phenol in the media increases by (10, 50,100,250,500,750 and 1000) ppm the time for degradation also increases. At higher initial concentrations, phenol takes more time to degrade completely [Arutchelvan et al., (2006)]. 100 ppm and 500ppm of phenol degrades in less time i.e. in (15-17) hours and (32-34) hours respectively compared to 750 and 1000 ppm of phenol which takes comparatively longer time to degrade i.e. (58-60) hours and (70-72) hours for complete degradation. The reason behind this increase in degradation time in case of 750 ppm and 1000 ppm of phenol is the inhibiting effect of phenol at higher substrate concentration 500 ppm. Saravanan et al., (2008) reported that microorganisms did not show any inhibitory effect and almost no lag phase was observed during its growth between 100 mg/l and 500 mg/l. Similar results were reported by Khazi et al., (2007) and Bandyopadhyay et al., (2000) the inhibiting effect of phenol as substrate was found to be predominant above the concentration of 600 ppm and 500 ppm respectively. Increasing phenol concentration also inhibits the growth of microbes that is reflected by the greater time taken for the complete degradation of phenol. It is also interesting to find that with the increase in the concentration of phenol the lag phase of the microbe also increases. The most probable reason behind the extended lag phase is due to the increasing toxicity of phenol with increase in concentration.

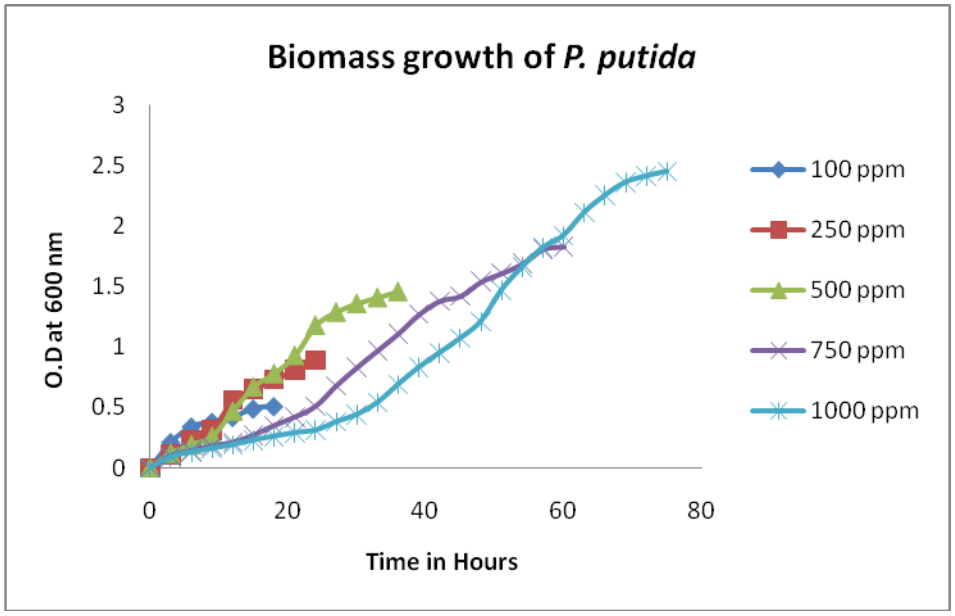

Fig. 19: Biomass growth of *P. putida* at different initial phenol concentration at optimum conditions: temp 30^0C, pH 7, 12 hours old 5% inoculum volume, shaking at 130rpm.

Biomass growth of *P. putida* was studied under optimum conditions and at different concentration of phenol. O.D of the broth was taken at regular intervals of time at 600nm. As shown in fig. 19 at higher phenol concentration of 750 ppm and 1000 ppm *P. putida* exhibited an extended lag phase due to toxic effect of phenol on the growth of the microorganisms. Lag phase has been observed up to 30 hours and thereafter up to 70 hours it follows log phase. Further it becomes stationary. At each of the initial phenol concentrations there was a period of lag phase where the substrate consumption was slow which later becomes faster in the exponential growth period and further becomes stationary and growth slows down which confirms that the substrate was completely consumed. Saravanan et al., (2008) studied the biomass growth at high concentration of phenol and reported that the time required to reach stationary phase goes on increasing as the initial phenol concentration increases. Similarly Bajaj et al., (2009) reported that the lag phase of growth was longer at increasing initial phenol concentrations. The increase in lag time depended on the inoculums size and on the inhibition constant. Saravanan et al., (2008) reported that the time required to reach stationary phase goes on increasing as the initial phenol concentration increases.

4.10. Conformational test for both the microorganisms responsible for simultaneous phenol degradation and Cr (VI) reduction

Table 3: Conformational result of simultaneous phenol degradation and Cr (VI) reduction

	Test 1			**Test 2**			**Test 3**	
Time in hrs		**Cr(VI) (mg/l)**	**Phenol (mg/l)**		**Cr(VI) (mg/l)**	**Phenol (mg/l)**	**Cr(VI) (mg/l)**	**Phenol (mg/l)**
0		5	250		5	250	5	250
6		4.99	249.98		4.98	198	4.98	249.98
12		4.98	249.95		4.96	102	4.91	249.86
18		4.93	249.88		4.91	32	4.86	249.79
24		4.91	249.83		4.88	0	4.73	249.73

	Test 4			**Test 5**			**Test 6**	
Time in hrs		**Cr(VI) (mg/l)**	**Phenol (mg/l)**		**Cr(VI) (mg/l)**	**Phenol (mg/l)**	**Cr(VI) (mg/l)**	**Phenol (mg/l)**
0		5	250		5	0	5	250
6		2.8	249.98		4.98	0	3.8	198
12		0	249.86		4.96	0	1.4	102
18		0	249.81		4.91	0	0	32
24		0	249.77		4.88	0	0	0

(Test 1 without any of the culture, **Test 2** with *P. putida* without *E. coli*, **Test 3** with *E. coli* without *P. putida*, **Test 4** with glucose and *E. coli* without *P. putida*, **Test 5** with both *P. putida* and *E. coli* but no phenol, **Test 6** both *P. putida* and *E. coli* with phenol as a carbon source**)**

Tests were carried out to investigate the simultaneous removal of phenol and Cr(VI) in seven different set of experiments and the results are listed in the above Table. Cr(VI) reduction along with phenol degradation occurred only in the presence of both *P. putida* and *E. coli* when phenol was used as the sole source of carbon. The concentration of Cr(VI) decreased from 5mg/L to 0 mg/L after (15-18) hours of incubation with 250 mg/L phenol as the sole added carbon source **(Test 6).** Little or no Cr(VI) reduction and phenol degradation was observed in the standards where no bacterial culture was inoculated (**Test 1**). Simultaneous Cr(VI) reduction and phenol degradation was also not observed when a single microbe i.e *E. coli* or *P. putida* was used (**Test 2 and 3**). Very less or no Cr(VI) reduction occurred when both the organisms were used but in the absence of phenol in the media.(**Test 5**). Whereas Cr(VI) reduction was observed when *E. coli* with glucose as electron donor was used without *P. putida* (**Table 4**). Phenol degradation was observed when *P. putida* alone was incubated in

the media (**Table 2**). This indicated that Cr(VI) reduction and phenol degradation were accomplished through biological activity of both the organisms. Similar work has been reported by Yun-guo et al., (2008) in which Cr(VI) reduction was insignificant in the absence of Cr(VI) reducing *Bacillus sp,* while phenol could be degraded by *P. putida Migula* in the same culture. So, it can be concluded that the metabolites formed from phenol degradation by phenol degrading bacteria acts as a electron donor for Cr(VI) reduction by the chromium reducing bacteria.

4.11. Effect of Cr (VI) concentration on phenol degradation and vice versa.

The effect of chromium on phenol degradation has been studied. The biological phenol degradation is greatly affected by the initial concentration of Cr(VI). The rate of phenol degradation declined with increasing the initial Cr(VI) concentrations. Low Cr(VI) concentration does not have much effect on phenol degradation whereas on increasing the concentration of chromium above an optimum limit reduces phenol degradation efficiency. Therefore higher Cr(VI) concentrations have an inhibiting and toxic effect on bacterial mechanism. Similar results were also reported by Yun-guo et al., (2008) where high Cr(VI) concentration above 5 ppm had inhibiting effect on phenol degradation. High concentration of Cr(VI) not only affects the significant phenol degradation, but also limits Cr(VI) reduction, due to the insufficient electron donors produced by phenol degradation. An increase in initial phenol concentration enhanced Cr(VI) reduction until some optimum value of phenol was reached. According to Nkhalambayausi-Chirwa and Wang, (2001), Phenol degradation results in the production and accumulation of a series of organic acid metabolites that interfere with the biological activity of the microbe and inhibits its Cr(VI) reduction capacity. Further high concentration of phenol also plays a major role in protein denaturation which itself inhibit the activity of the bacterial metabolism.

4.12. Simultaneous phenol and chromium degradation using mixed culture of microorganisms

Different Cr(VI) concentrations i.e (5, 10 and 15) ppm were subjected to degradation in the presence of 250 ppm and mixed culture of microorganisms.

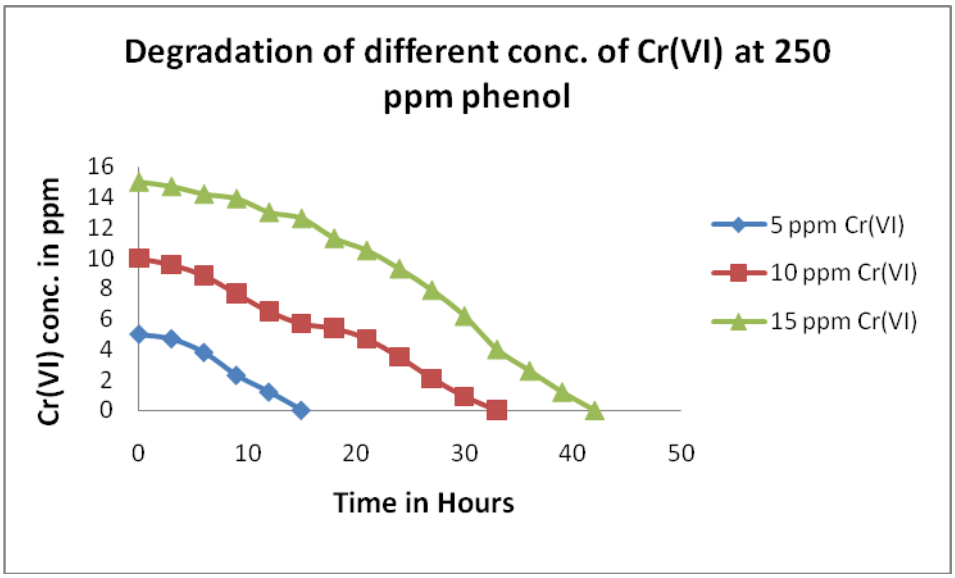

Fig. 20: Degradation of varying concentrations of Cr(VI) in the presence of 250 ppm of phenol at optimum conditions.

From the above graph it is observed that in the presence of 250 ppm phenol, lower concentration of Cr(VI) i.e. 5 ppm degrades completely in (12-15) hours whereas higher concentration of Cr(VI) 10 ppm and 15 ppm takes prolonged time i.e (30-32) hours and (40-42) hours respectively for their complete degradation in the presence of 250 ppm of phenol. This is because of the insufficient or less amount of energy and electron donor produced during phenol degradation by *P. putida* which was inadequate for higher concentrations of Cr(VI). Results obtained are similar to those of Yun-guo et al., (2008).

Similarly degradation of 250 ppm of phenol was studied at various concentrations of Cr(VI) as shown in Fig. 21.

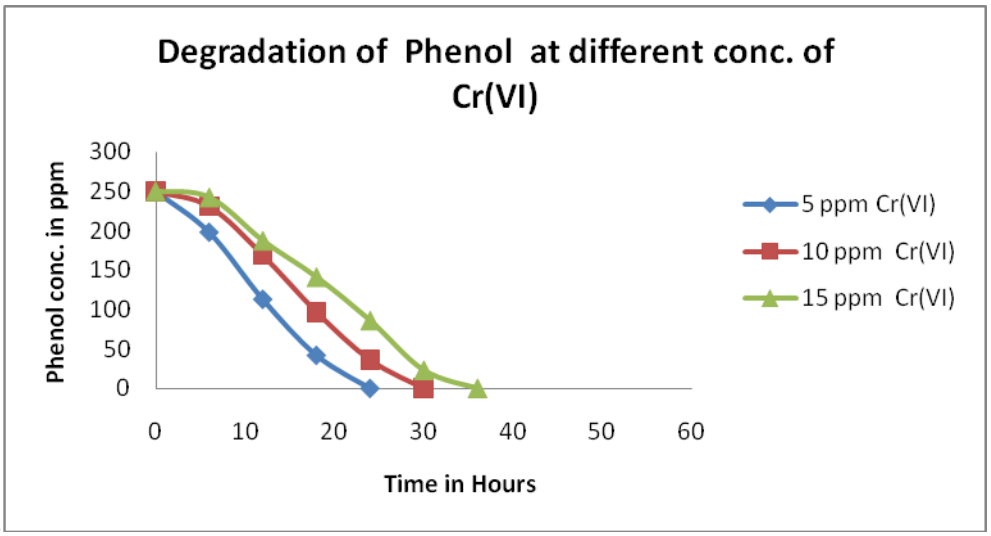

Fig. 21: Degradation of 250 ppm of phenol at various initial concentrations of Cr(VI) at optimum conditions.

From the above graph it can be inferred that 250 ppm phenol takes longer time for degradation i.e. (28-30) hours and (34-36) hours in the presence of 10 ppm and 15 ppm Cr(VI) respectively. Whereas 250 ppm of phenol degrades in (22-24) hours in the presence of lower concentrations of Cr(VI) i.e. 5 ppm. This is because of the inhibitory effect of high Cr(VI) concentration on phenol degradation

Biomass for both the organisms at (5, 10 and 15) ppm of Cr(VI) in the presence of constant phenol concentration of 250 ppm was also studied. Biomass was studied in terms of OD taken at 600nm.

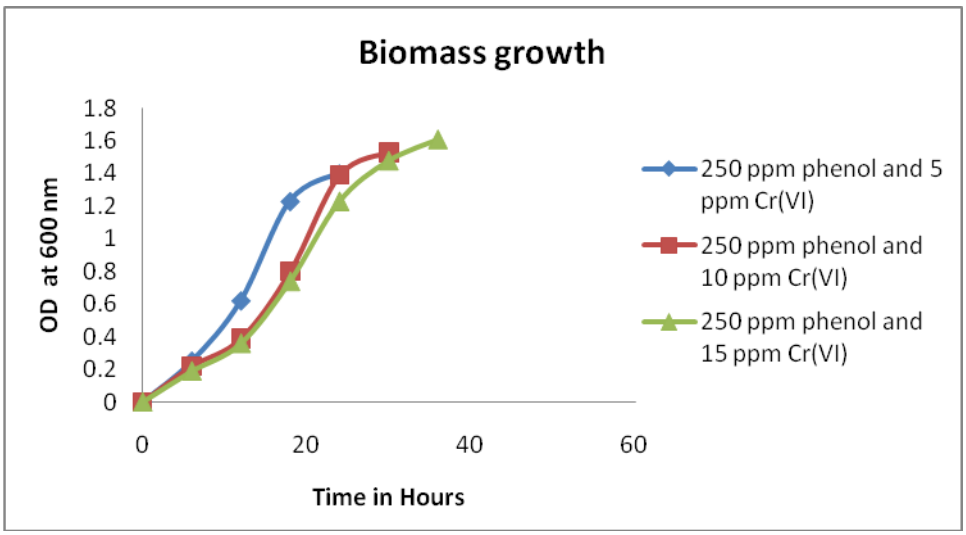

Fig. 22: Biomass growth of mixed microbial culture during degradation of varying concentrations of Cr(VI) at constant phenol concentration

Different Cr(VI) concentrations i.e (5, 10 and 15) ppm were degraded in the presence of 500 ppm of phenol.

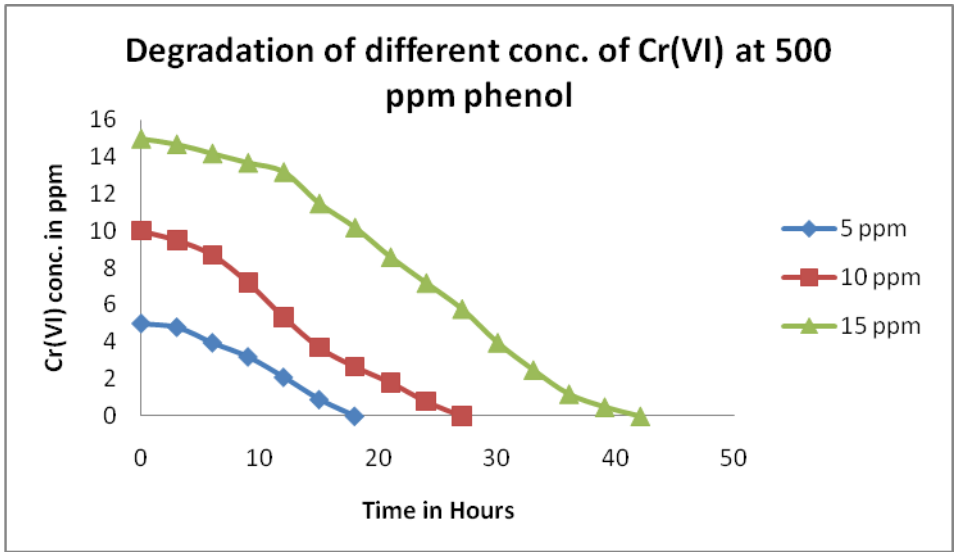

Fig. 23: Degradation of varying concentrations of Cr(VI) in the presence of 500 ppm of phenol at optimum conditions.

As shown in the graph above 5 ppm of Cr(VI) takes less time i.e (16-18) hours for complete degradation while 10 ppm and 15 ppm of Cr(VI) take longer time for their complete degradation. Thus it can be understood that the energy and electron produced by *P. putida* during phenol degradation were sufficient for degradation of 5 ppm of Cr (VI) by *E. coli.* The inhibitory effect of high concentration of phenol on Cr(VI) reduction can also be inferred from the fig. 23. 15 ppm of Cr (VI) took more than 42 hours for its complete degradation when present with 500 ppm of phenol.

Similarly degradation of 500 ppm of phenol was studied at various concentrations of Cr(VI) as shown in Fig. 24

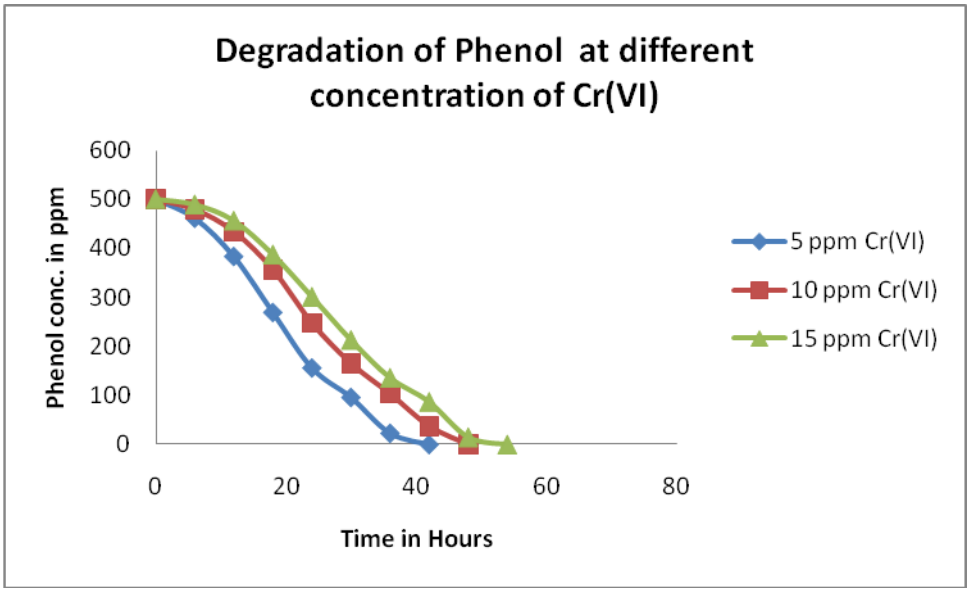

Fig. 24: Degradation of 500 ppm of phenol at various initial concentrations of Cr(VI) at optimum conditions.

Fig. 24 shows the degradation of 500ppm of phenol in the presence of various concentrations of Cr(VI). Degradation of phenol i.e. 500ppm is greatly affected by the presence of different concentration of Cr(VI) due to the inhibitory effect of Cr(VI) on phenol degradation. From the above graph it can be observed that 500 ppm phenol in the presence of (5, 10 and 15) ppm Cr(VI) gets degraded at (40-42) hours, (46-48) hours and (52-54) hours respectively.

Biomass for both the organisms at (5, 10 and 15) ppm of Cr(VI) in the presence of constant phenol concentration of 500 ppm was also studied. Biomass was studied in terms of OD taken at 600nm.

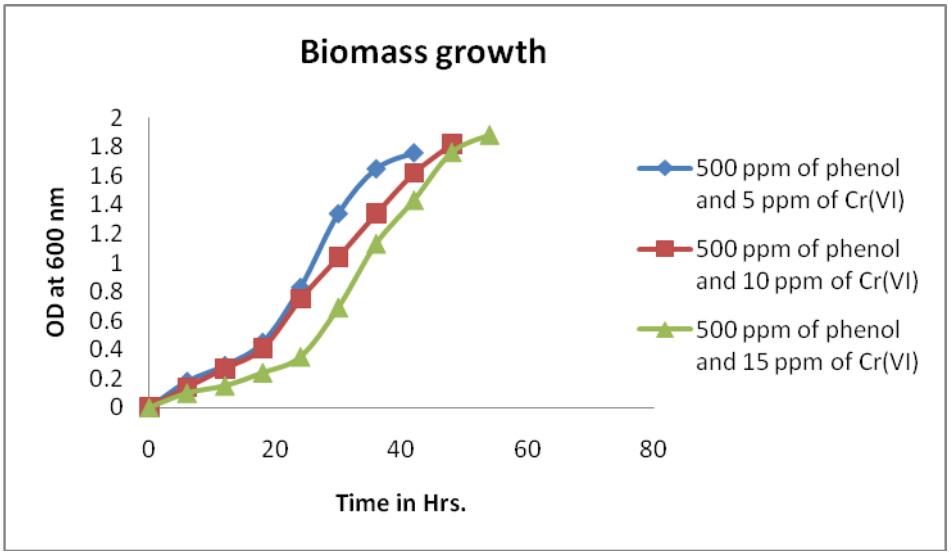

Fig. 25: Biomass growth of mixed microbial culture during degradation of varying concentrations of Cr(VI) at constant phenol concentration

Different Cr(VI) concentrations i.e (5, 10 and 15) ppm were degraded in the presence of 750 ppm of phenol.

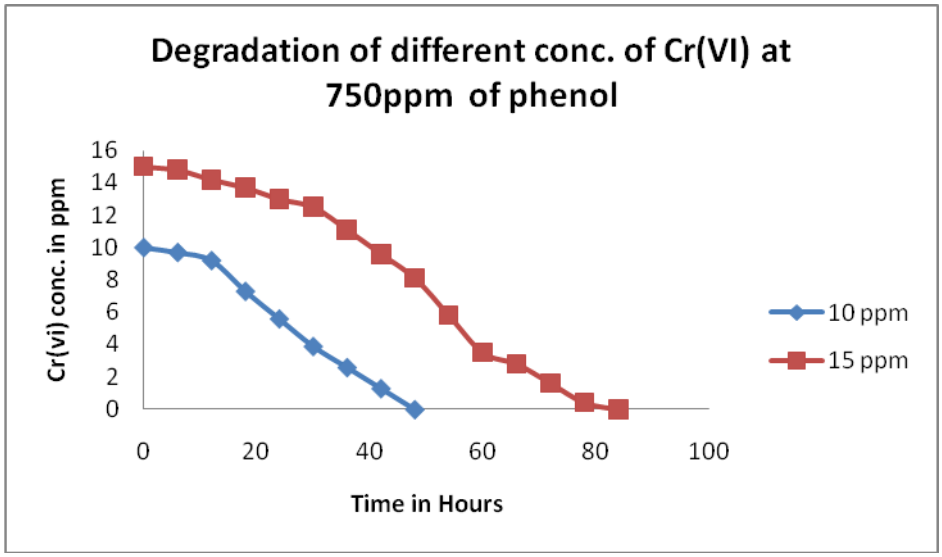

Fig. 26: Degradation of varying concentrations of Cr(VI) in the presence of 750 ppm of phenol at optimum conditions.

From the above graph it is observed that 10 ppm of Cr(VI) is degraded in (46-48) hours while 15 ppm of Cr(VI) gets degraded in more than 80 hours. This is due to the toxic and inhibitory effect of high concentration of phenol on Cr(VI) reduction. From the above different combinational studies it can be concluded that 15 ppm Cr(VI) takes longer time for degradation in presence of 750 ppm phenol as compare to the time taken for the degradation in the presence 250 ppm and 500 ppm of phenol. Similarly degradation of 750 ppm of phenol was studied at various concentrations of Cr(VI) as shown in Fig. 27.

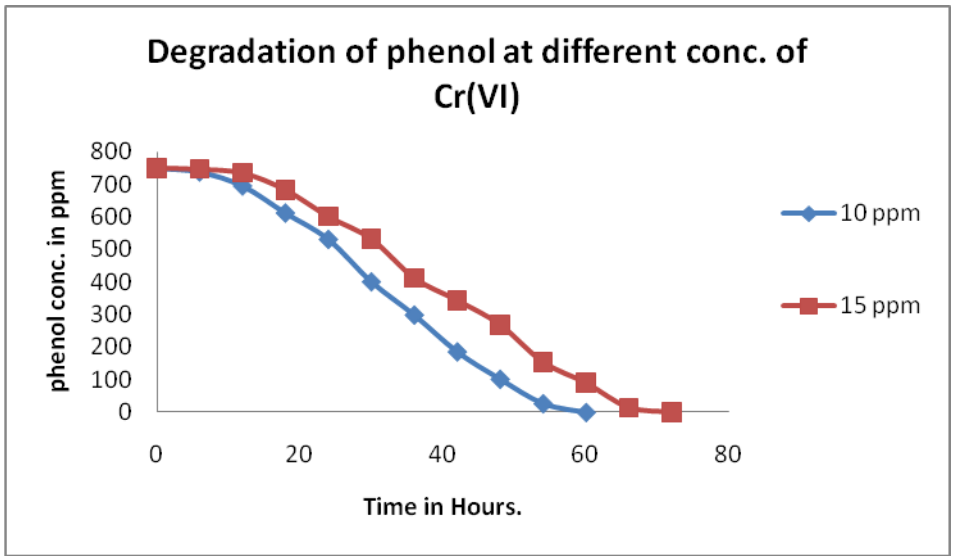

Fig. 27: Degradation of 750 ppm of phenol at various initial concentrations of Cr(VI) at optimum conditions.

As shown in the figure above degradation of 750 ppm was greatly affected by the presence of different concentration of Cr(VI), this is basically due to the inhibitory effect of higher concentration of Cr(VI) on phenol degradation. From the above graph it can be observed that 750 ppm phenol in the presence of 10 ppm and 15 ppm Cr(VI) gets degraded in (58-60) hours and (70-72) hours respectively.

Biomass OD at 600nm was studied for both the microorganisms in the culture containing 10 ppm and 15 ppm of Cr(VI) respectively along with 750 ppm phenol.

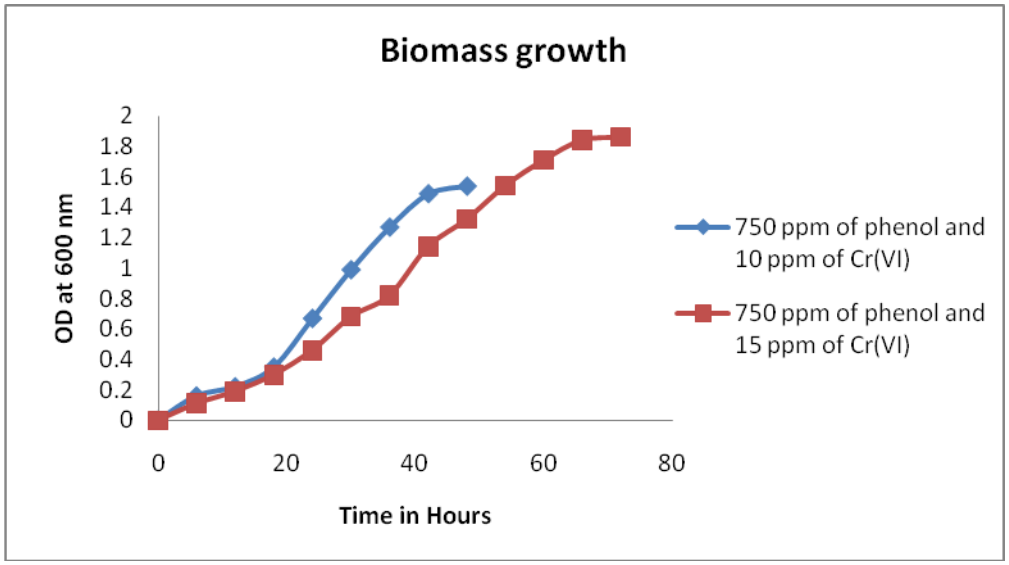

Fig. 28: Biomass growth of mixed microbial culture during degradation of varying concentrations of Cr(VI) at constant phenol concentration

Degradation of different concentration of phenol in the presence of constant Cr(VI) of 5ppm was also studied. As shown in the fig. 29 various concentrations of phenol (10, 50,100,250,500,750 and 1000) ppm were subjected to degradation in the presence of constant Cr(VI) concentration of 5 ppm has and a mixed culture of microorganisms is inoculated. Here phenol is used as the carbon source by *P. putida* and electron donor for Cr(VI) degradation by *E. coli*.

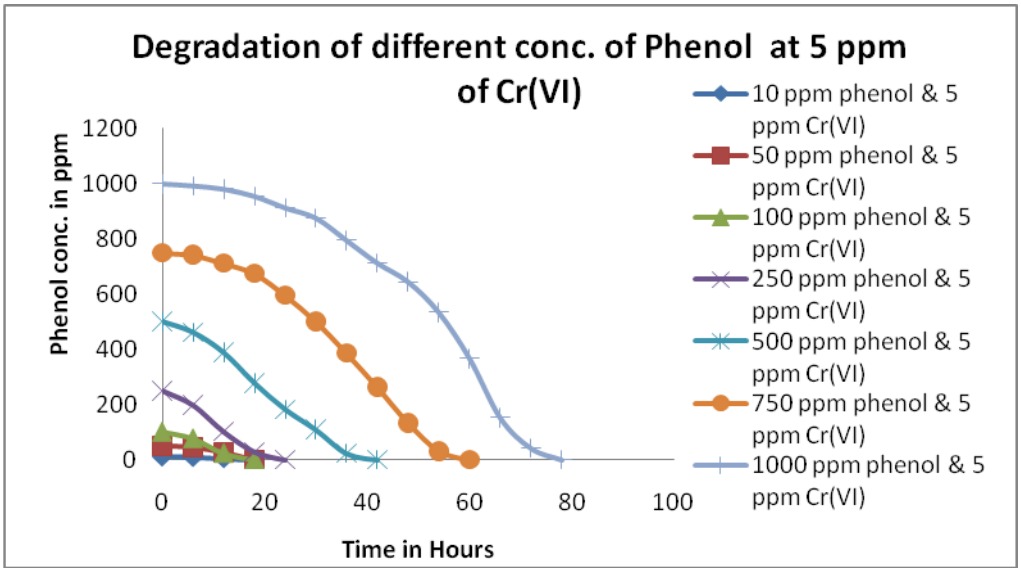

Fig. 29: Degradation of varying concentrations of phenol in the presence of constant 5 ppm of Cr(VI) at optimum conditions.

From the above graph it is observed that in the presence of 5 ppm of Cr(VI), the time of degradation increases with the increasing concentrations of phenol. Thus, in the presence of 5 ppm of Cr(VI), 10 ppm phenol takes nearly 12 hours for complete degradation whereas 1000 ppm phenol takes around (76-78) hours for its complete degradation. Maximum Phenol degradation is achieved at a phenol concentration of 500 ppm. Beyond this concentration the trend of degradation changes with an increase in the lag phase. The degradation initially slows down when high initial concentration of phenol are considered, but later becomes faster and follows similar degradation trend till the complete degradation of phenol is achieved. It is attributed to the toxicity of phenol that increases with increasing phenol concentration and it inhibits the growth of microbes that is reflected in greater time taken to degrade completely. Yun-guo et al., (2008) reported similar result on the effect of phenol concentration on the Cr(VI) reduction. A phenol concentration over a range of 25 mg/L to 250 mg/L and an initial Cr(VI) concentration of 15 mg/L were considered for the study. Chirwa et al., (2000) also examined similar work and observed optimum Cr(VI) reduction at a phenol concentration of 200 mgl^{-1} and an initial Cr(VI) concentration of 2 mgl^{-1}. While complete phenol degradation was observed only in cultures under low initial Cr(VI) concentration ($\leq$10 mgL^{-1}). Experimental results also indicated the *E. coli* utilizes metabolites formed from phenol degradation as electron donors for Cr(VI) reduction. Cr(VI) inhibited both Cr(VI) reduction and phenol degradation when Cr(VI) concentration exceeded the optimum value (20 mg/L), whereas phenol enhanced both Cr(VI) reduction and phenol degradation at a optimal value of 100ppm. Tziotzios et al., (2008) also investigated the effect of phenol concentrations on the rates of phenol removal and Cr(VI) reduction under constant initial Cr(VI) concentration of about 5.5 ppm and phenol concentration ranging from 350 ppm to 1500 ppm. Optimum phenol degradation was achieved at lower Cr(VI) concentration and simultaneously maximum Cr(VI) reduction was achieved at lower initial phenol only. Cr(VI) reduction rate of 0.062 g Cr(VI) L^{-1} d^{-1} and phenol removal rate of 3.574 g phenol L^{-1} d^{-1} was observed at the initial phenol concentration of 500 mg phenol L^{-1} and 5.5 mg L^{-1} of Cr(VI). Song et al in 2009 have investigated the simultaneous reduction Cr(VI) and phenol degradation by using a single culture of *Pseudomonas aeruginosa* CCTCC AB91095. The metabolites formed during phenol degradation were found to be used as energy source for Cr(VI) reduction by *P. aeruginosa*. Phenol degradation ranging from 10 ppm to 200 ppm in the presence of 2 ppm Cr(VI) and also 2 ppm of Cr(VI) reduction in the presence of different initial concentration of phenol from 10 ppm to 200 ppm were studied. At higher concentration of phenol the rate of Cr(VI) reduction decreased due to the excess formation of

organic acid metabolites which inhibit the chromium reduction. Simultaneously at higher concentration of Cr(VI) the phenol degradation rate was decreased due to the toxic effect of high concentration of Cr(VI) on phenol degradation.

Study of reduction of 5 ppm of Cr(VI) by *E. coli* in the presence of different concentration of phenol ranging (10,50,100,250,500,750 and 1000) ppm. As shown in the (figure 30), 5 ppm Cr(VI) takes longer time for reduction i.e. (16-18) hours and (14-15) hours in the presence of 10 ppm and 50 ppm of phenol respectively. In the presence of 100 ppm of phenol, Cr(VI) gets reduced in a short time span of (10-12) hours. This decrease in time of reduction is probably due to the energy and electron donors produced from phenol degradation by *p. putida* that is sufficient in case of 100 ppm of phenol than in case of 10 ppm and 50 ppm. With further increase in the concentration of phenol in the culture the time for reduction of 5ppm of Cr(VI) by *E. coli* also goes on increasing. The toxic and inhibitory effect of high concentration of phenol is the basic reason for the increase in time for Cr(VI) reduction. Similar studies were reported by other researchers [Yun-guo et al., (2008) and Song et al., (2009)].

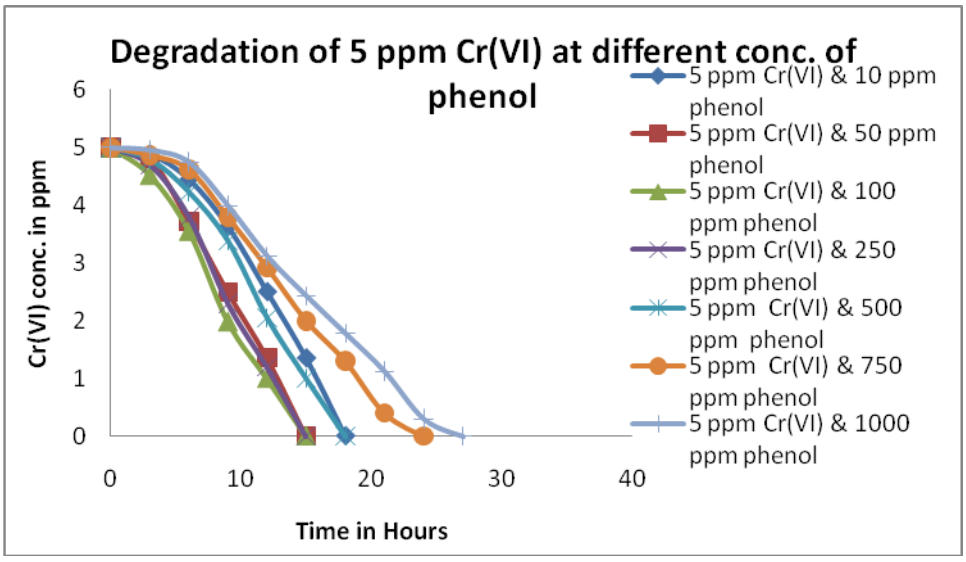

Fig. 30: Degradation of Cr(VI) at various concentration of phenol in presence of 5ppm Cr(VI) at optimum conditions

Biomass study was also carried out for both the microbes in the presence of 5 ppm of Cr(VI) and varying phenol concentration of (10,50,100,250,500,750 and 1000) ppm.

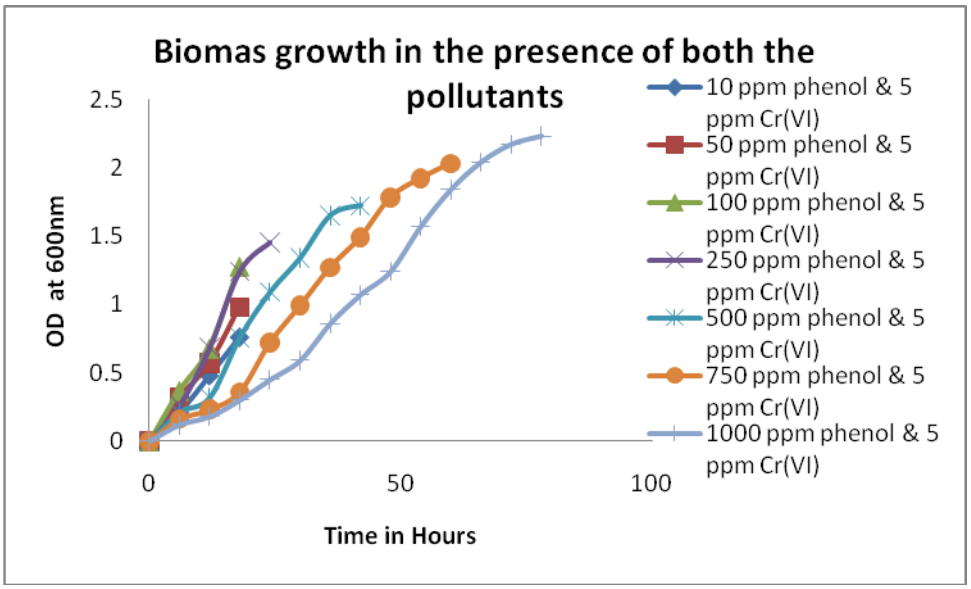

Fig. 31: Biomass growth of mixed culture of microorganisms during degradation of different concentration of phenol at 5 ppm Cr(VI) concentration at optimum conditions

4.13. Analysis under 100X microscope

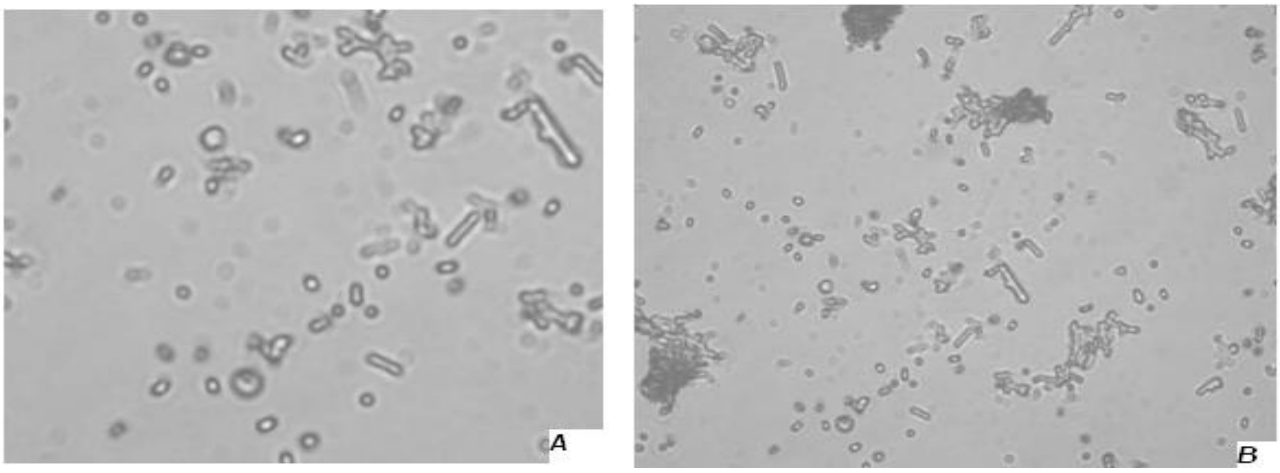

Fig. 32 (A): 100x microscopic view of mixed culture of microbes showing *P. putida* and *E. coli*
Fig. 32 (B): 100x microscopic view of Secondary metabolites produced during the degradation process

The 100x microscopic view of mixed culture of microbes (*P. putida* and *E. coli*) is shown in Fig. 32 (A). Fig. 32 (B) shows the 100x microscopic view of the microbes along with the secondary metabolites produced during the degradation proscess. As seen in the figure clumps of

microorganisms are surrounded by black cloudy mass.These cloudy masses are the secondary metaboltes produced during the degradation proscess.

4.14. Resistant to other heavy metals

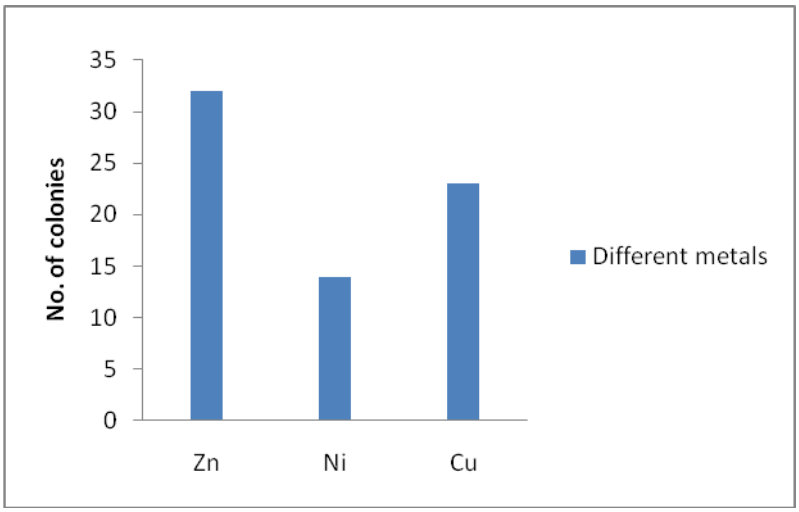

Fig. 33: Heavy metal resistance studies and relative growth of mixed culture of microorganisms in the presence of various concentrations of heavy metals on nutrient agar media.

The inhibitory effect of aerobic phenol degradation was studied in the presence of various concentrations of heavy metals. Similarly Pyo, (2003) reported the efficient removal of 1000ppm of phenol in 30 hours in the presence of other heavy metals like mercury, cadmium, copper and zinc. Maximum tolerance level of other metals by the mixed culture of microorganisms present in the medium along with phenol and chromium were also checked. Mixed culture of the organisms were allowed to grow on agar plate containing heavy metals Zn, Cu, Ni each present at a concentration of 10mg/l. Number of colonies were counted after incubation of 48 hours with both the microorganisms. As shown in the fig. 33 highest numbers of colonies were observed on the agar petriplate containing Zinc followed by Cu and Ni. The number of colonies achieved in case of Zn was 32 colonies followed by 25 colonies in case of Cu and 14 colonies in case of Ni. From the result obtained it was confirmed that the heavy metals have less inhibitory effect on the microbial growth and degradation efficiency. Deeb et al., (2009) also studied phenol degradation by *P. putida* in the presence of other heavy metals like Cd^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+} which were used as $CdCl_2$, $CuCl_2.2H_2O$, $NiCl_2$ and $ZnCl_2$, respectively, in various concentrations ranging from 0.001-8 mM. The plates were incubated at 28°C to 30°C for (72-96) hours and it was found that in the presence of other heavy metals no significance difference was observed in phenol degradation. El-Naas et

al., (2009) suggested that the activity and ability of bacteria for phenol degradation is greatly affected in the presence of other contaminants in the refinery wastewater. The Chemical analysis indicated that the presence of major contaminants, including sulphates and heavy metal ions such a like iron, aluminium and zinc had negligible effect on the biodegradation rate of phenol. Wasi et al.,(2008) have isolated a Pseudomonas fluorescence SM1 isolate which was found to be resistant to some major water pollutants namely Cd^{2+}, Cr^{6+}, Cu^{2+}, Ni^{2+}, Pb^{2+} and phenols up to a concentration four times to the normal levels occurring in the highly pullulated regions. These strains were found to efficiently degrade both high concentration of phenol and Cr(VI).

4.15. Bioreactor study

Fig. 34: Experimental set up (Bioreactor, New Brunswick, BIO FLOW 410)

Simultaneous Degradation of phenol and reduction of Cr(VI) using mixed culture of microorganisms in the bioreactor. Large scale degradation of normal and industrial effluent at its optimum conditions in a bioreactor (New Brunswick, BIO FLOW 410) was performed using the microbial strains of phenol degrading bacteria *Pseudomonas putida* (MTCC 1194) and Chromium reducing bacteria *Escherichia coli* (NCIM 5051).

4.16. Physico-chemical characteristic of industrial effluent as studied using bioreactor

The industrial effluent was collected from the local textile industry and the physico-chemical parameters were estimated using standard methods. Chromium and copper are usually found in the dyeing and finishing section and phenol with other heavy metals are mostly found in the printing, bleaching, washing section of the textile industry.

Table 4: Showing the Physico-chemical characteristic of textile industry effluent before and after treatment and their permissible limits

Parameters	EFFLUENT (UNTREATED)	TREATED	STANDARD
COD (mg/l)	1340	246	200-300
BOD (mg/l)	530	138	100-150
TOC (mg/l)	207	81	50-100
TDS (mg/l)	2630	96	100
TSS (mg/l)	320	45	100
pH	8.2	7	7
PHENOL (mg/l)	250	0	0.05-0.1
Cr(VI) (mg/l)	5	0	0.05-0.1
TOTAL Cr (mg/l)	5	1.459	2
Zn (mg/l)	10	3.632	5
Cu (mg/l)	8	2.302	3
Ni (mg/l)	7	2.594	3

4.17. Phenol degradation study using bioreactor

The degradation of 250 ppm phenol in the presence of 5 ppm Cr(VI) was also studied in a bioreactor under optimized conditions.

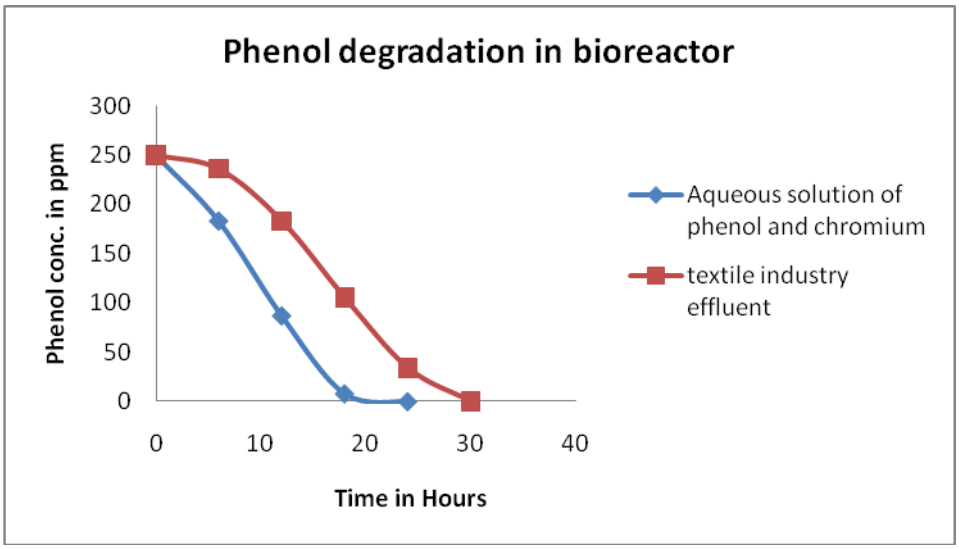

Fig. 35: Degradation of 250 ppm of phenol in bioreactor in the presence of 5 ppm of Cr(VI) at optimum conditions: agitation 200 rpm, pH 7, temperature 30^0C, DO 100 ppm.

As shown in the fig. 35 under optimized conditions when an aqueous solution of 250 ppm phenol and 5 ppm Cr(VI) is subjected to degradation 250 ppm of phenol it took nearly (18-20) hours for its complete degradation. Whereas when the textile industry effluent was subjected to degradation of 250 ppm of phenol, it took nearly (28-30) hours for complete degradation of the two pollutants. The reason behind the prolonged degradation is the presence of other heavy metals and pollutants in the industrial effluent which extends the time required to degrade the phenol. Presence of other heavy metal thus slightly influences the phenol degradation due to the inhibition effect of the other heavy metals on the microorganisms.

4.18. Cr(VI) degradation study using bioreactor

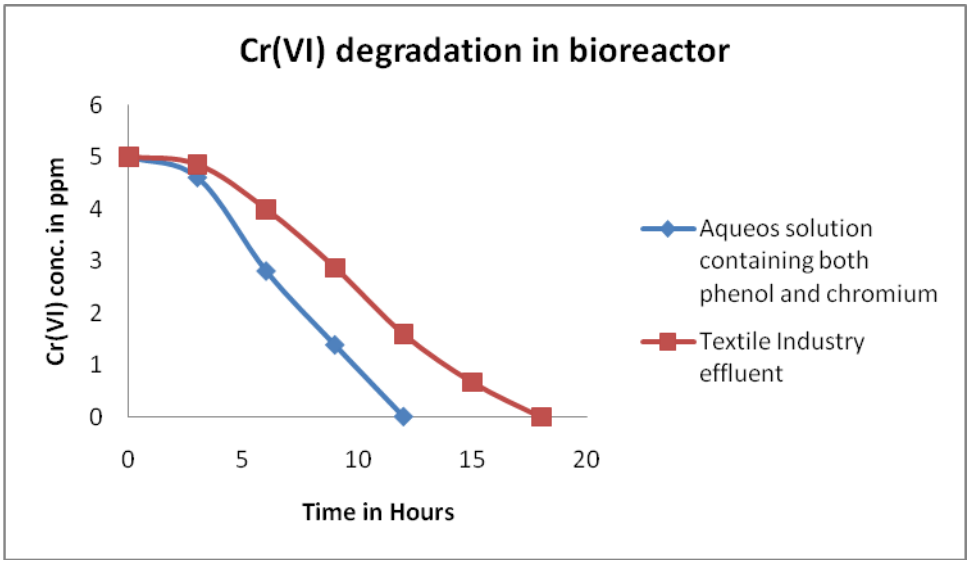

Fig. 36: Degradation of 5 ppm of Cr(VI) in bioreactor in the presence of 250 ppm of phenol at optimum conditions: agitation 200 rpm, pH 7, temperature 30^0C, DO 100 ppm

Similarly Cr(VI) reduction was also carried out in bioreactor. As shown in the above Fig. 36 when aqueous solution of 5 ppm Cr(VI) and 250 ppm phenol was subjected to degradation under optimized conditions 5 ppm of Cr(VI) took less time i.e. (10-12) hours for its complete degradation. Whereas it took (16-18) hours for its complete degradation when present in the textile industry effluent. The reason behind the prolonged time of degradation was due to the presence of other heavy metals and organic and inorganic pollutants in the textile industry effluent this reduced the degradation rate.

4.19. Simultaneous degradation of phenol & Cr(VI) in industrial effluent in a bioreactor

Large scale degradation of aqueous solution of phenol with chromium and textile industry effluent was performed at its optimum conditions in a bioreactor (New Brunswick, BIO FLOW 410). All process parameters in the bioreactor like temperature, pH, DO and addition of anti foam was controlled automatically and remained constant throughout the operation as shown in fig. 37.

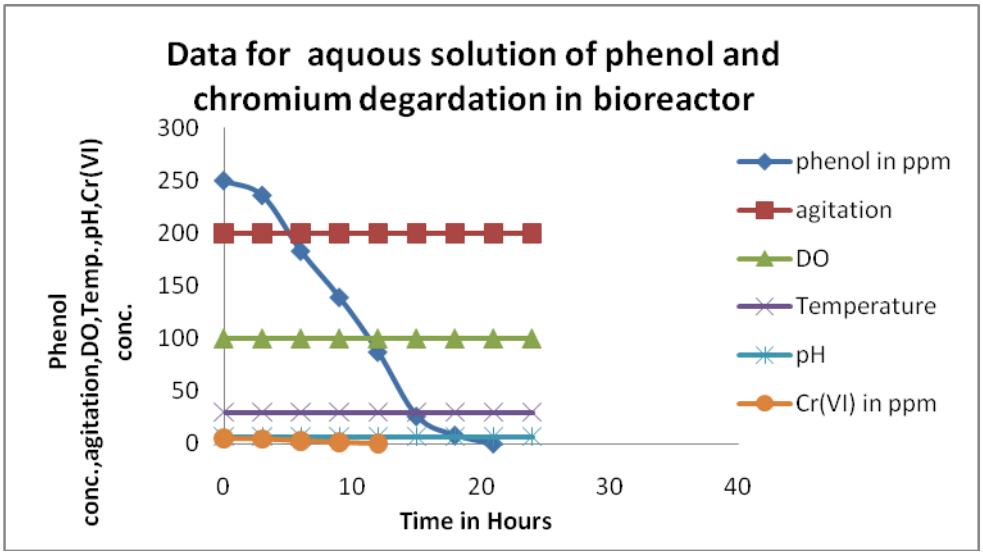

Fig. 37: Degradation of 250 ppm of Phenol and 5 ppm of Cr(VI) in aquous solution at optimum conditions: agitation 200 rpm, pH 7, temperature 30^0C, DO 100 ppm

The results of the study of degradation of the aqueous solution of phenol and Cr (VI) in a bioreactor indicate that complete removal of phenol was achieved within (18-20) hours and chromium within (10-12) hours. The other experimental parameters were maintained constant like pH 7 temperature 30^0C, agitation 200 rpm, DO 100 mg/L etc. The degradation time for both phenol and Cr(VI) was observed to be decreased when studied in bioreactor rather than in shake flask.

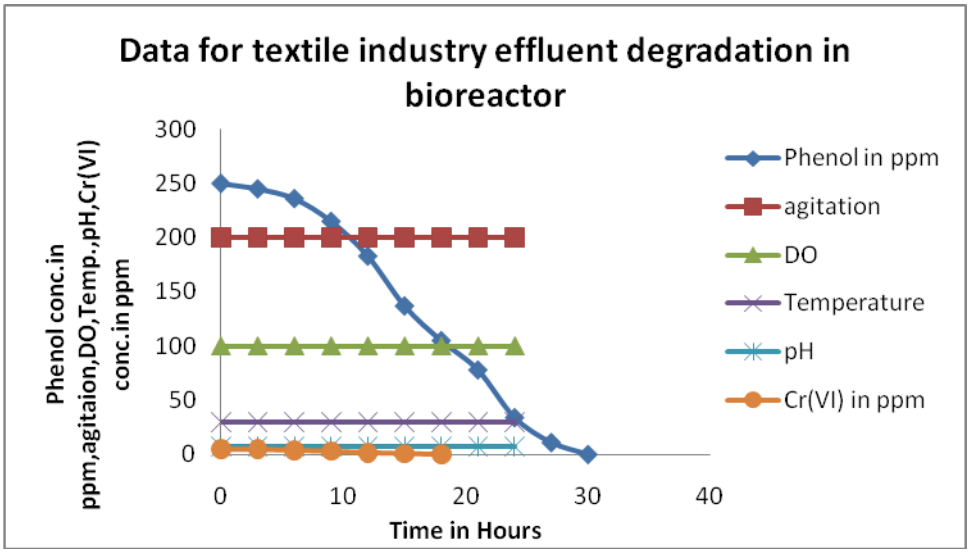

Fig. 38: Degradation of 250 ppm of Phenol and 5 ppm of Cr(VI) in textile inustry effluent at optimum conditions: agitation 200 rpm, pH 7, temperature 30^0C, DO 100 ppm.

The results of the treatment of textile industry effluent indicate that all water parameters including the concentration of phenol and chromium along with other heavy metals is reduced and brought to their permissible limits. The complete removal of phenol was achieved in (28-30) hours and chromium within (16-18) hours. The other experimental parameters were maintained constant like pH 7 temperature 30^0C, agitation 200 rpm, DO 100 mgl/L etc. The degradation time for phenol and Cr(VI) was observed to increase when present in an textile industry effluent rather than in aqueous solution due to the presence of other heavy metals and organic and inorganic pollutants in the textile industry effluent.

4.20. SEM and EDX analysis

4.20.1. SEM

Scanning Electron Microscopy-Energy Dispersive X-ray Spectroscopy analysis (SEM-EDX) is evaluated to understand the cell morphology, elemental composition of both the untreated and treated industrial effluent. Detailed examination by SEM helped us to detect the morphological and topological changes that occurred in the microbial cells before and after degradation. Similarly the industrial effluent was also examined using EDX for the conformation of the pollutants and the heavy metals before and after treatment.

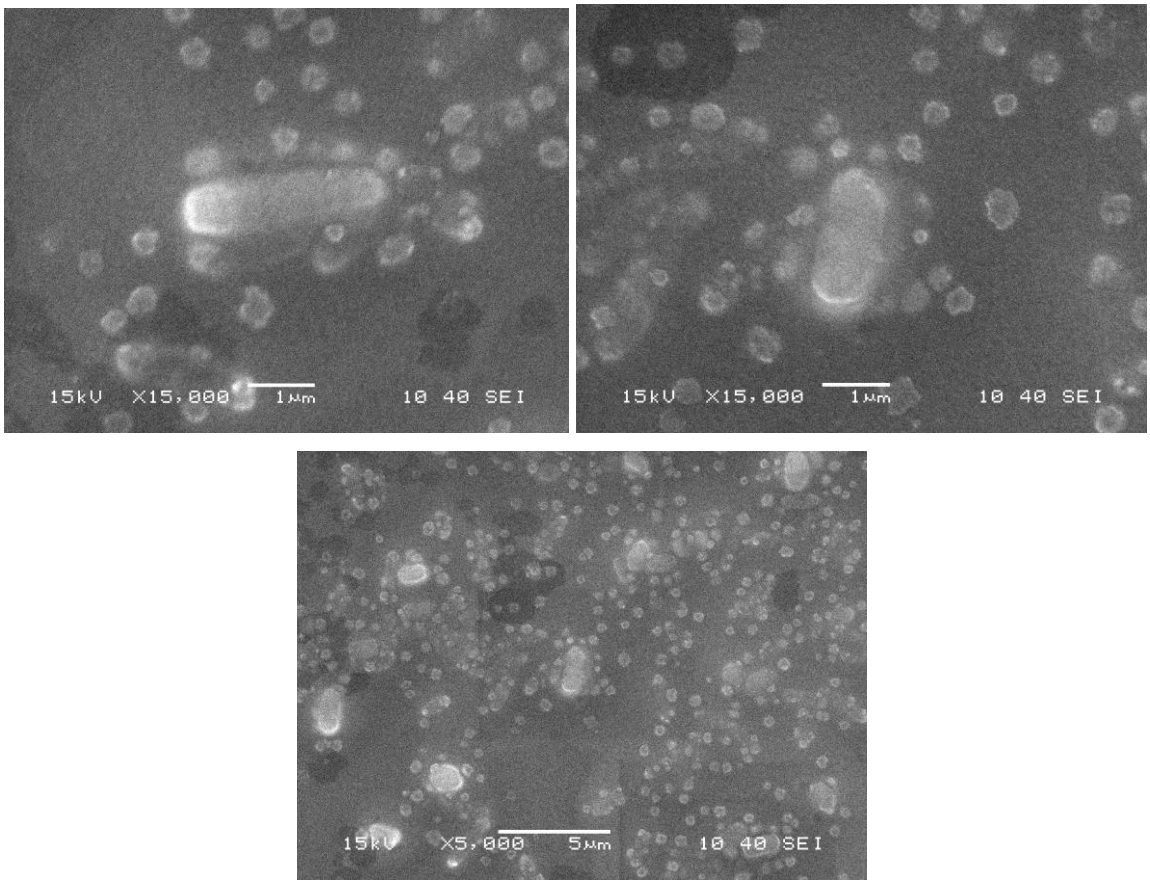

Fig. 39: SEM image of individual and both *E. coli* and *P. putida* before treatment

Microbial cell image of individual organisms i.e, *Escherichia coli* and *Pseudomonas putida* were studied under SEM. Both the microbes are rod shaped and size of *E. coli* is slightly larger than *P. putida*. Chen et al 2007 also achieved similar SEM image of *Pseudomonas putida* BCRC 14349 grown on chitosan bead in their paper that is similar to our study.

Ackerley et al., (2006) also studied the structural morphology of *Escerichia coli* K-12 before and after chromate stress. It was clearly observed in the *E. coli* cell. When the cell was suddenly exposed to chromate it becomes elongated compared to the normal cells due cellular oxidative stress of chromium on the non adapted cells of *E. coli.*

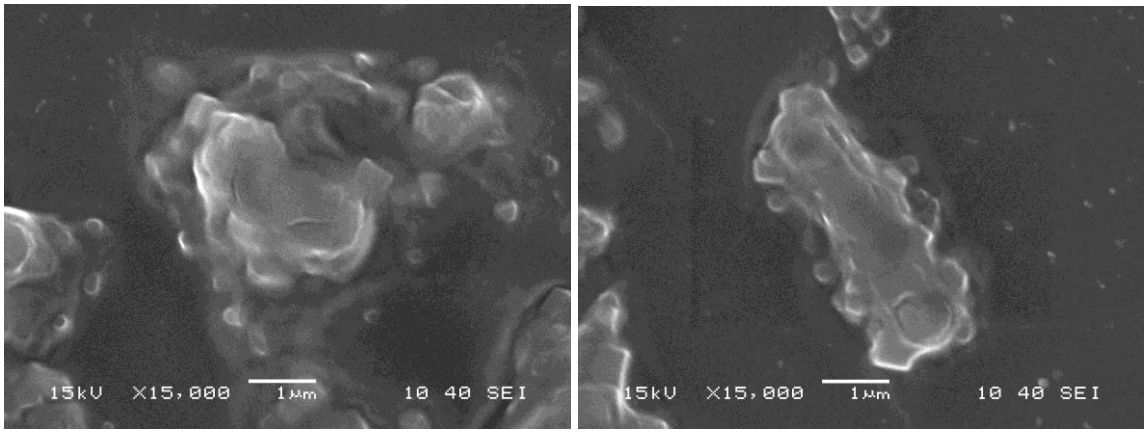

Fig. 40: SEM image of individual microorganisms *Escherichia coli* and *Pseudomonas putida* after degradation.

A number of cytological and morphological changes have been observed in microbial cells in the presence of Phenol and chromium. Throughout the years most of the study has been done on yeasts especially *Candida* sp. [Kappeli et al., (1978)]. Also there is a limited study reported for *Acinetobacter* species [Kennedyet and Finnerty., (1975)]. But not much work has been reported on the structural changes in their cellular morphology of *Pseudomonas putida and Escherichia coli.* The cell wall disruption can be clearly seen in the above image due to the stress of toxic metals and organic and inorganic pollutants. The cell was also found to be swollen and the cell size is increased after the uptake of the pollutants and heavy metals our result was similar to the findings of [Cameotra and Singh, (2009)]. Adheration of the heavy metals can be seen on the surface of the cell of the microbe. Some extra cellular secretion can also be seen in the above image which is likely to be the secondary metabolites produced after degradation. Due to chromium and other heavy metals stress some elongation and deformation of the cell can also be seen. Similar result was reported by Francisco et al., (2010). This indicates that on long term exposure to the toxic pollutants the microbial cells undergo cell lysis. Cr and other heavy metal deposition on cell surface were visualized. Xie et al., (2010) also studied the SEM micrograph of *E. cloacae* taken before and after Cr(VI) loading. The morphology, physiological and biochemical characteristics of the strain results was similar as our result. The presence of heavy metal was clearly reflected on the cell

morphology and the cells were observed to have a rough cell wall and an elongated shape. The secretion of the metabolites was also reported in their study.

4.20.2. EDX of treated effluent

The industrial effluent sample was subjected to ENERGY DISPERSIVE X-RAY analysis to find its elemental composition before and after biological degradation. As shown in the Fig. 41 various pollutants like Cr, Zn, Ni and Cu along with other elements like Na, C and Oxygen were present in the untreated textile industry effluent. Whereas as seen in Fig. After treatment the EDX of biologically degraded sample confirms the complete removal of the Cr along with other heavy metals. Only few unused media components like Mg, Na, K, S, Cl were found after complete removal of the pollutants.

Similar finding was observed by Srivastava et al., (2007) using *Acinetobacter sp*. for the removal of Cr (VI) from tannery effluent. Various authors like Francisco et al., (2010), Li et al., (2008) and Das and Mishra, (2008) have also studied the presence of chromium with other heavy metals by EDX in their study.

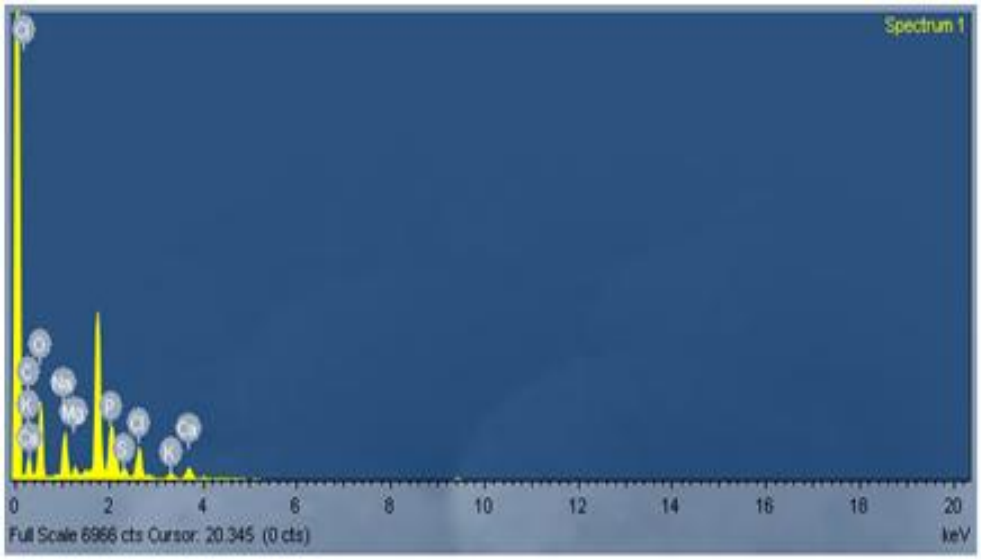

Fig. 41: EDX of treated textile industry effluent

4.21. Total chromium analysis

The culture medium containing 250 ppm phenol and 5 ppm Cr(VI) was also analyzed by atomic absorption spectroscopy technique to find the total chromium at different time

intervals in the culture medium. Degradation of both the pollutants had been done with the help of the mixed culture of microorganisms. The Atomic Absorption Spectroscopy reading showed that Cr(VI) gradually reduces to Cr(III) and some part of it is getting degraded completely. The total chromium analysis result indicates that after degradation the total chromium remaining in the aqueous solution is 1.529 ppm at 18 hours.

Table 5: Total Chromium analysis at different intervals of time

Time in hour	**Total Chromium (mg/l)**
3	4.495
6	3.760
9	3.012
12	2.399
15	1.532
18	1.529

Few researcher have studied the removal of chromium and other heavy metals from textile industry effluent and analysed the amount of pollutant that present before after degradation by atomic absorption spectrophotometer (AAS). Halimoon and Yin, (2010) studied the removal of heavy metals from textile wastewater using zeolite, the amount of heavy metals like Cr, Cu, Pb present in textile industry effluent was calculated with the help of AAS. Similarly, Yusuff and Sonibare, (2004) studied the amount of heavy metal (aluminium, chromium, iron, zinc, manganese, and copper) present in the textile industry effluent using AAS. Benazir et al., (2009) and Sundar et al., (2010) also estimated total chromium by AAS to know the total chromium present in tannery effluent after degradation by the microbial consortia.

5. CONCLUSIONS

Phenol and chromium are two major pollutants being discharged from various industries during mechanical operations. They mix in the water bodies and make them unusable. Textile industry present in Rajahmundry, AP, India is one of the example of such industries which is polluting the nearby water bodies and lands as well as the whole environment. People are facing various health problems by being exposed to the polluted water. Our primary objective is to degrade the contaminants present in the effluent and make them usable. Hence the present investigation is based on the simultaneous removal of phenol and chromium which are the major contaminant of the textile industry effluent in the presence of a mixed culture of indigenous microorganisms i.e phenol degrading strain of *Pseudomonas putida* MTCC(1194) and chromium reducing strain of *Escherichia coli* NCIM(5051). The influence of various operational and growth parameters like carbon and nitrogen source, volume and age of inoculums, pH, temperature are optimized to make them more efficient to completely degrade the pollutants present in the textile industry effluent.

The key features of the present investigation are summarized as follows

Sample collection and analysis

- Industrial effluent was collected from a local textile industry situated in Rajahmundry, AP, India and was subjected to physico-chemical characterization. Presence of BOD, COD, TOC, TDS, TSS, pH, colour and odour were observed.
- The EDX result shows the presence of Cr, Zn, Cr, Ni and other organic contaminants in the form of C and O.
- Standard Chemical assay for phenol and chromium determination was performed to find out the actual concentration. The amount of other heavy metals present is determined with the help of AAS. The analysis result shows the presence of two major pollutants 250 mg/L of phenol and 5 mg/L of chromium along with other heavy metals Zn (10mg/L), Cr (8mg/L) and Ni (7 mg/L) etc.

Biodegradation of phenol and chromium

- The phenol degrading strain of *Pseudomonas putida* MTCC(1194) and chromium reducing strain of *Escherichia coli* NCIM(5051) were obtained from MTCC Chandigarh and NCIM Pune respectively.
- Individual phenol degradation by *Pseudomonas putida* MTCC(1194), and chromium reduction by *Escherichia coli* NCIM(5051) was studied .
- Simultaneous removal of phenol and chromium using co-culture of indigenous microorganisms was performed.

Optimization of the culture growth condition

- Maximum biomass productivity along with phenol and chromium degradation by the individual microorganism in the presence of different carbon and nitrogen source is studied.
- The result shows for both the microorganisms dextrose is the optimum carbon source and peptone is the optimum nitrogen source.

Optimization of process parameters

- Effect of pH on phenol and chromium degradation by individual microorganisms shows the rate of phenol degradation by *Pseudomonas putida* and rate of Cr(VI) reduction by *E. coli* is maximum at pH 7.
- Influence of incubation temperature suggests the rate of phenol degradation by *P. putida* and rate of Cr(VI) reduction by *E. coli* is maximum at an optimum temperature of 30^0C.
- Influence of inoculum volume and inoculum age indicates maximum rate of phenol degradation and Cr(VI) reduction can be achieved by both of the microorganisms in 5% , 12 hour old inoculums.

Degradation study

- In shake flask under optimum process conditions *P. putida* is able to degrade a maximum of 1000 ppm of phenol in (70 –72) hours.
- Similarly at optimum process conditions *E. Coli* is able to completely degrade 40 ppm of Cr(VI) in (40 – 42) hours.
- Conformational test for both the microorganisms responsible for simultaneous phenol degradation and Cr(VI) reduction was performed.
- Simultaneous phenol degradation and chromium reduction by the mixed culture of microorganisms is studied by varying both phenol and chromium in the range of (250, 500 and 750) ppm of phenol and (5,10 and 15) ppm of Cr(VI) respectively.
- Degradation of various phenol concentration ranging from 10 ppm to 1000 ppm were studied in the presence of 5 ppm of Cr(VI) and result shows complete degradation of 1000 ppm of phenol is obtained at (76 – 78) hours.
- Simultaneously reduction of constant 5 ppm of Cr(VI) was observed at different higher concentration of phenol ranging from (10–1000) ppm. The result shows the 5 ppm of Cr(VI) took (24–26) hours for its complete reduction when present with 1000 ppm of phenol.
- Cell of mixed culture of organisms before and after degradation were studied under 100X microscope showing the metabolites production during the degradation process.
- Heavy metal resistance study for the mixed culture of microorganisms indicate the sequence (Zn > Cu > Ni) in this order.
- Degradation of both phenol and Cr(VI) was observed in a bioreactor under optimum process conditions for aqueous solution containing both the pollutants and for textile industry effluent.
- The bioreactor results shows 250 ppm of phenol and 5 ppm of Cr(VI) takes (18–20) hours and (10–12) hours for their complete degradation when present in aqueous solution containing both phenol and chromium.
- But in case of treatment of textile effluent in bioreactor the degradation of both phenol and Cr(VI) takes (28–30) hours and (16–18) hours respectively for their complete degradation indicating the inhibitory effect of organic and inorganic pollutants along with other heavy metals present in the textile industry effluent.

- The textile industry effluent after treatment was subjected to EDX conforms the complete removal of various heavy metals like (Cr, Zn, Ni).
- The treated effluent when subjected to analysis indicates very trace amount of heavy metals that attained the permissible limits [Zn-3.632 mg/L, Cu – 2.302 mg/L and Ni – 2.594 mg/L].
- From the results obtained from SEM analysis shows number of cytological and morphological changes like cell wall disruption, cell size elongation swelling of the cell, extracellular secretion in the microbial cells before and after degradation.
- Total chromium analyzed by AAS shows the gradual reduction of Cr(VI) to Cr(III) and simultaneous complete reduction of chromium at different time intervals.

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